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(54) Title: COISOGENIC EUKARYOTIC CELL COLLECTIONS

(57) Abstract: Collections of cultured eukaryotic cells, particularly human cells, in which the cells are coisogenic at a common target locus, are provided. Particularly provided are collections of coisogenic cells that differ in genomic sequence by no more than 0.05%, excluding changes at the target locus, collections in which the coisogenic cells differ in genomic sequence by no more than 0.005%, excluding changes at the target locus, and collections in which the cells jack heterologous genetic elements within 10 kilobases of the coisogenic target locus. Kits comprising the cell collections, methods of making the collections, kits for making the collections, and methods of using the collections to facilitate pharmacogenomic analyses are presented. Preferred target loci at which the cells are coisogenic include genes that affect drug resistance, drug sensitivity, and/or drug metabolism.

## Coisogenic Eukaryotic Cell Collections

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application serial no. 60/325,992, filed September 27, 2001, the disclosure of which is incorporated herein by  
5 reference in its entirety.

### FIELD OF THE INVENTION

The present invention is in the field of molecular biology, and relates to coisogenic eukaryotic cell collections and methods of use therefor. More specifically, the invention relates to collections of eukaryotic cells that have been engineered to differ from  
10 one another by as few as one encoded amino acid at a defined target locus, particularly, but not exclusively, target loci that encode proteins that affect responsiveness to therapeutic agents, and to pharmacogenomic methods based thereupon.

## BACKGROUND OF THE INVENTION

The newly-emerging field of pharmacogenomics is premised on the notion that statistical correlations of genotypic variations that occur naturally within a population (allelic variation) with their respective phenotypes can be used to predict an individual patient's responsiveness to therapy based upon knowledge of the patient's genotype; the ultimate goal is to stratify patient populations into genetic cohorts for which therapy can be separately tailored. See, e.g., Adam *et al.*, "Pharmacogenomics to predict drug response," *Pharmacogenomics* 1(1):5-14 (2000); Judson *et al.*, "The predictive power of haplotypes in clinical response," *Pharmacogenomics* 1(1):15-26 (2000).

As a preliminary to any such clinical prognostication, naturally occurring alleles must be identified and the alleles correlated with observable clinical phenotypes. A sufficient number of individuals must be studied for the correlations to achieve statistical reliability. Each of these requirements limits the utility of current pharmacogenomic approaches.

Although the first of these limitations is being addressed, in part, by public, quasi-public and private undertakings to identify all common single nucleotide polymorphisms (SNPs) in the human genome (see, e.g., NCBI's dbSNP database at <http://www.ncbi.nlm.nih.gov/SNP/>; the Karolinska Institute's Human Genic Bi-Allelic Sequences Database at <http://hgbase.cgr.ki.se/>; and the SNP Consortium's database at <http://snp.cshl.org/>), patients carrying uncommon, perhaps unique, alleles will remain outside the prognostic scope of such analyses. Furthermore, the requirement for observable clinical phenotypes and the requirement for patient populations of adequate statistical size are not addressed by the simple expedient of cataloguing common SNPs.

One clinical phenotype that has been proposed for pharmacogenomic-based prognostication is multidrug resistance. See, e.g., Kerb *et al.*, "ABC drug transporters: hereditary polymorphisms and pharmacological impact in MDR1, MRP1 and MRP2," *Pharmacogenomics* 2(1):51-64 (2000); Szakacs *et al.*, "Diagnostics of multidrug resistance in cancer," *Pathol. Oncol. Res.* 4(4):251-7 (1998).

Genetic polymorphisms in proteins other than the multidrug transporters are also known to play a role in drug sensitivity and in drug resistance. For example, the

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cytochrome 450 enzyme encoded by CYP2D6 is known to metabolize as many as 20% of commonly prescribed drugs. The gene is highly polymorphic in the population; certain alleles result in the poor metabolizer phenotype, characterized by a decreased ability to metabolize the enzyme's substrates.

5                   *In vitro* assays have been developed to assess the drug sensitivity of individual cells. For example, U.S. Patent Nos. 6,277,655 and 5,872,014 describe assays specific for activity of the multidrug transporter ABCB1 (MDR1), as does Ludescher *et al.*, *Br. J. Haematol.* 82(1):161-8 (1992). See also, "In vitro assays for chemotherapy sensitivity," *Crit. Rev. Oncol. Hematol.* 15(2):99-111 (1993); Cree *et al.*, "Tumor  
10 chemosensitivity and chemoresistance assays," *Cancer* 78(9):2031-2 (1996); Apoptosis and Cell Proliferation, 2<sup>nd</sup> ed., Boehringer Mannheim, 1998 (available on-line at [http://biochem.boehringer-mannheim.com/prod\\_inf/manuals/cell\\_man/acp.pdf](http://biochem.boehringer-mannheim.com/prod_inf/manuals/cell_man/acp.pdf)), and Poirier (ed.), Apoptosis Techniques and Protocols, Humana Press, 1997 (ISBN: 0896034518).

                  Although the *in vitro* drug resistance (equally and conversely, drug  
15 sensitivity) phenotype of individual cells can at times predict the clinical phenotype of the entire organism, to apply such *in vitro* assays to pharmacogenomic analyses requires the *in vitro* assay of cells bearing different alleles of the gene or genes of interest. Few such alleles are available in cell lines that can readily be assayed, and when available, are often present on genetically disparate backgrounds.

20                   Recently, there have been efforts to create collections of cell lines that have defined genetic modifications on a uniform genetic background for use in various *in vitro* assays.

                  Genetic modifications that have typically been contemplated for eukaryotic cells used in screening assays include targeted deletion or disruption of genes, dominant  
25 negative suppression of gene expression, and change in gene copy number. See, e.g., U.S. Patent Nos. 5,569,588, 5,777,888, 6,165,709, 6,046,002. For the most part, the preferred organism for such genetic modification has been yeast, notably *Saccharomyces cerevisiae*, due in part to its ability to support homologous recombination at efficiencies far greater than those possible in mammalian cells. Where the cell line is mammalian,  
30 however, often the chosen modification leaves heterologous nucleic acids at or near the

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target locus, a legacy of virally-mediated modification events. See, e.g., U.S. Patent No. 6,207,371.

Thus, there exists a need for methods that would more readily permit pharmacogenomic analyses without requiring the prior large scale correlation of naturally-  
5 occurring alleles with naturally-occurring, clinically observable phenotypes. There is a further need in the art for collections of eukaryotic cells, particularly mammalian cells, that have defined mutations in target loci, particularly mutations that recapitulate naturally-occurring alleles, on a uniform genetic background. There is a particular need for collections of eukaryotic cells that lack heterologous nucleic acid insertions additional to the  
10 targeted changes. In particular, there exists a need for such cell collections having targeted mutations in genes that affect drug resistance.

#### SUMMARY OF THE INVENTION

The present invention satisfies these and other objects in the art by providing, in a first aspect a collection of cultured cells, comprising at least 5, 10, or at least  
15 25 genotypically distinct cells, wherein each of the genotypically distinct cells is coisogenic with respect to the others in the collection at a common target locus. The genotypically distinct cells of the collection are separately assayable.

As used herein, two genotypically distinct cells are "coisogenic" with respect to one another if derived from a common ancestor cell and engineered to differ from  
20 one another in genomic sequence at a predetermined target locus. The genomic sequence differences at the target locus must be sufficient to alter the amino acid sequence encoded at the target locus by at least one amino acid. The term "coisogenic" permits of changes as between the genomes of the genotypically distinct cells additional to the changes at the target locus.

25 In certain preferred embodiments, the coisogenic cells of the collection are "exceptionally coisogenic", that is, differ in genomic sequence by no more than 0.05%, excluding changes at the target locus, or "perfectly coisogenic", differing in genomic sequence by no more than 0.005%, excluding changes at the target locus. In certain preferred embodiments, the cells are alternatively, or additionally, legacy-free, that is,

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lacking in heterologous genetic elements within 10 kilobases of any codon of the target locus.

The coisogenic cells can be from any eukaryote; although usefully mammalian, especially human, the cells can also be of yeast or plant origin.

5 In certain embodiments, the genotypically distinct cells of the collection collectively include each of the 20 natural amino acids at a single residue encoded at the target locus. In other embodiments, the genotypically distinct cells collectively include a predetermined amino acid at each residue encoded after the initiator methionine at the target locus. In particularly preferred embodiments, the genotypically distinct cells  
10 collectively include at least one, and on occasion a plurality, of naturally occurring allele of the target locus.

The cells of the collection can further comprise a common selectable marker at a genomic locus different from said target locus, and/or a marker unique to said genotypically distinct cell, the unique marker being at a locus different from the target locus.

15 The target locus can be any locus of interest, and in particularly useful embodiments, is selected from the group of loci affecting drug resistance (sensitivity) or drug metabolism consisting of: CYP1A2, CYP2C17, CYP2D6, CYP2E, CYP3A4, CYP4A11, CYP1B1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP11A, CYP2C19, CYP2F1, CYP2J2, CYP3A5, CYP3A7, CYP4B1, CYP4F2, CYP4F3, CYP6D1, CYP6F1,  
20 CYP7A1, CYP8, CYP11A, CYP11B1, CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, CYP51, ABCB1, ABCB4, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, MRP7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, EPHX1, EPHX2, LTA4H, TRAG3, GUSB, TMPT, BCRP, HERG, hKCNE2, UDP glucuronosyl transferase (UGT), sulfotransferase, sulfatase, glutathione S-transferase (GST) -alpha, glutathione S-  
25 transferase -mu, glutathione S-transferase -pi, ACE, and KCHN2.

In another aspect, the invention provides the coisogenic cell collection in the form of a kit. The kit comprises at least five genotypically distinct cells, the cells contained within separate, structurally discrete, fluidly noncommunicating containers, wherein each of the genotypically distinct cells is coisogenic with respect the others at a  
30 target locus common thereamong; the structurally discrete containers are commonly packaged.

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In some embodiments, the kit further comprises a computer readable medium, recorded upon which is a dataset (typically, a relational database) that describes the target locus genotype of each of said genotypically distinct cells.

In another aspect, the invention provides a method of making a coisogenic  
5 cell collection.

In its most basic form, the method comprises collecting at least 5 genotypically distinct cells, each of the genotypically distinct cells being coisogenic with respect to the others at a target locus common thereamong, into a collection in which each of the genotypically distinct cells can be separately assayed.

10 Typically, the coisogenic cells will first be prepared, and the method will thus further comprise the antecedent step of engineering, into at least four of five cultured cells, the cells having derived from a common eukaryotic ancestor cell, a genomic sequence alteration at a target locus common thereamong. For purposes of the present invention, the sequence alterations should be sufficient to cause at least five distinct protein  
15 sequences collectively to be encoded by the cells at the target locus.

In preferred embodiments, the engineering is effected by introducing a targeting oligonucleotide into each of said at least four cultured cells. The targeting oligonucleotide effects site-specific change to the cellular genomic DNA. Alternatively, in a multistep process, a targeting oligonucleotide is first used to effect a change in a genomic  
20 recombination-competent substrate, such as an artificial chromosome, and the recombination-competent substrate then introduced into each of the four cultured cells.

In another aspect, the invention provides a kit useful for creating the coisogenic cell collections of the present invention. The kit comprises at least four targeting oligonucleotides of distinct sequence; and a eukaryotic cell. The targeting oligonucleotides  
25 are sufficient to effect four different sequence changes, each sequence change sufficient to alter the protein sequence, at the target genomic locus.

The coisogenic cell collections of the present invention can be used for multiplex, including high throughput multiplex screening for mutations that affect a cellular phenotype in vitro.

30 Thus, in another aspect, the invention provides a method of identifying genotypes of a target locus that alter a cellular phenotype, comprising a first step of

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assaying each genotypically distinct cell of a coisogenic cell collection for a common phenotypic characteristic. The genotypically distinct cells are coisogenic at the target locus, preferably exceptionally or perfectly coisogenic, and/or legacy-free. After assay, the method calls for identifying from the assay results at least one cell having an altered phenotypic characteristic; and correlating, for the cell or cells with altered phenotypic characteristic, the results of said phenotypic assay with the cell's target locus genotype. Such correlation of phenotypic assay results with target locus genotype identifies genotypes of the target locus that alter the cellular phenotype.

Usefully, the phenotypic characteristic can be responsiveness of the cell to a xenobiotic, and the method can thus include the antecedent step of contacting the coisogenic cell collection with a xenobiotic. In certain embodiments of the method, the cells of the collection are coisogenic at a target selected from the group consisting of: CYP1A2, CYP2C17, CYP2D6, CYP2E, CYP3A4, CYP4A11, CYP1B1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP11A, CYP2C19, CYP2F1, CYP2J2, CYP3A5, CYP3A7, CYP4B1, CYP4F2, CYP4F3, CYP6D1, CYP6F1, CYP7A1, CYP8, CYP11A, CYP11B1, CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, CYP51, ABCB1, ABCB4, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, MRP7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, EPHX1, EPHX2, LTA4H, TRAG3, GUSB, TMPT, BCRP, HERG, hKCNE2, UDP glucuronosyl transferase (UGT), sulfotransferase, sulfatase, glutathione S-transferase (GST) -alpha, glutathione S-transferase -mu, glutathione S-transferase -pi, ACE, and KCHN2.

The correlations can thereafter optionally be collected into at least one dataset, typically one or more relational databases, usefully recorded on a computer-readable medium.

In a further aspect, the invention provides a method of predicting a phenotypic characteristic of a cell based upon its genotype at a target locus. The method comprises using the cell's genotype at the target locus, or a unique identifier thereof, as a query to retrieve from a dataset data that report a correlated phenotypic characteristic, wherein the dataset includes such correlations for at least five cells that are coisogenic at the target locus; the retrieved phenotypic characteristic provides a prediction of the cell's phenotypic characteristic.



The above and other objects and advantages of the present invention will be apparent upon consideration of the following detailed description.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

5 Unless otherwise made explicitly clear by context, the indefinite article "a" intends one or more of the objects referenced immediately thereafter.

As used herein, the term "cell" intends a eukaryotic cell. Unless otherwise made explicitly clear by context, the singular term "cell" equally intends a plurality of genetically identical cells, such as a plurality of cells from a clonal eukaryotic cell line. A  
10 "cultured cell" is a eukaryotic cell (or clonal eukaryotic cell line) that is maintained alive *in vitro* in nutrient media, or that has previously been propagated *in vitro* in nutrient media for at least one doubling.

"Genotypically distinct" cells have nonidentical genomic sequences.

A "target locus" is a genomic region that includes all exons of an  
15 expressed protein.

As used herein, two genotypically distinct cells are "coisogenic" with respect to one another if derived from a common ancestor cell and engineered to differ from one another in genomic sequence at a predetermined target locus. The genomic sequence differences at the target locus must be sufficient to alter the amino acid sequence encoded  
20 at the target locus by at least one amino acid. The term "coisogenic" permits of changes as between the genomes of the genotypically distinct cells additional to the changes at the target locus.

"Exceptionally coisogenic" cells are coisogenic cells that differ in genomic sequence by no more than 0.05%, excluding changes at the target locus.

25 "Perfectly coisogenic" cells are coisogenic cells that differ in genomic sequence by no more than 0.005%, excluding changes at the target locus.

Cells, or genetic alterations, therein are said to be "legacy-free" if lacking in heterologous genetic elements within 10 kilobases of an engineered genomic sequence

alteration. When used with respect to coisogenic cells, the cells are legacy-free if lacking in heterologous genetic elements within 10 kilobases of any codon of the target locus.

As used herein, "**heterologous genetic elements**" are sequences of greater than 25 consecutive nucleotides that derive from – and that can thus be shown to  
5 be present in – species different from that from which the coisogenic cells derive; heterologous genetic elements thus include, *inter alia*, all genetic elements derived from prokaryotic cells, including prokaryotic genomic DNA; genetic elements derived from prokaryotic episomes, including fertility factors; genetic elements derived from bacteriophage; as well as genetic elements from eukaryotic viruses.

10 As used herein, the term "**collection**", as applied to cells, intends that the cells are in sufficient spatial proximity to one another as readily and contemporaneously to be subject to the same experimental protocol. The term "**library**" is intended to be synonymous with "collection" in all respects.

As used herein, the term "**xenobiotic**" intends a foreign compound  
15 introduced into a biological system, such as an inorganic or organic compound foreign to the cell or organism under study, or a compound naturally present in the cell or organism under study but administered by nonnatural routes or at unnatural concentrations.

#### Coisogenic eukaryotic cell collections, methods of making, and methods of use

The present invention is made possible by our recent discovery of methods  
20 and compositions, to be described in further detail below, for creating site-specific mutations in genomic DNA of eukaryotic cells, including mammalian cells, at efficiencies and with a precision not hitherto achievable using homologous recombination or earlier approaches based upon oligonucleotide-mediated gene repair.

The methods permit point mutations to be targeted with high efficiency to  
25 genomic DNA incubated in cellular extracts, such as artificial chromosomes incubated in cellular extracts, and also permit mutations to be targeted with high efficiency directly into the chromosomes of cultured cells. The efficiency is sufficiently high as to obviate the concomitant insertion of selectable markers or other exogenous DNA, permitting cells with defined mutations to be created legacy-free. These methods permit us readily to create

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collections of coisogenic eukaryotic cell lines, including legacy-free, perfectly coisogenic cell lines, that possess targeted and discrete changes at given target loci.

These collections of coisogenic cells have substantial utility in pharmacogenomic studies, obviating the identification of naturally-occurring allelic variants, observation of naturally occurring clinically-relevant phenotypes in a human population, and association of the naturally-occurring allelic variants with the naturally-occurring, clinically-relevant phenotypes. In embodiments particularly useful for pharmacogenomic studies, the target loci at which the collection of cells are coisogenic encode proteins known to affect drug resistance (conversely, drug sensitivity), and drug metabolism.

The collections of coisogenic cells have further utility in studies of the structure-activity relationships of existing, and of potential new, therapeutic agents, permitting multiplex analysis of the effects of amino acid changes on ligand-receptor interactions. The collections of coisogenic cells are also useful in screening for agonists and antagonists of proteins that affect drug resistance, sensitivity, and metabolism.

Thus, in a first aspect, the invention provides a collection of at least 5 genotypically distinct cells, typically as a collection of at least 5 genotypically distinct eukaryotic cell lines. Each of the genotypically distinct cells (or cell lines) is coisogenic to the others of the genotypically distinct cells (or cell lines) in the collection at a common target locus. In addition, each of the genotypically distinct cells can be separately assayed.

Given the generality of our oligonucleotide-mediated mutational approach, the cultured cells of the invention can be any eukaryotic cell amenable to *in vitro* culture.

Among mammalian cells, human cells have particular utility, particularly for pharmacogenomic uses. Also very useful, particularly for structure-activity studies, are cells from related primates, such as chimpanzee, monkeys (including rhesus macaque), baboon, orangutan, and gorilla, and those from rodents typically used as laboratory models, such as rats, mice, hamsters and guinea pigs. Cells can also usefully be from lagomorphs, such as rabbits; and from larger mammals, such as livestock, including horses, cattle, sheep, pigs, goats, and bison. Also useful are cells from fowl such as chickens, geese, ducks, turkeys, pheasant, ostrich and pigeon; fish such as zebrafish, salmon, tilapia, catfish, trout and bass; and domestic pet species, such as dogs and cats.

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Plant cells for which coisogenic cell collections can usefully be constructed according to the methods of the present invention include, for example, experimental model plants, such as *Chlamydomonas reinhardtii*, *Physcomitrella patens*, and *Arabidopsis thaliana*; crop plants such as cauliflower (*Brassica oleracea*), artichoke (*Cynara scolymus*);

5 fruits such as apples (*Malus*, e.g. *Malus domestica*), mangoes (*Mangifera*, e.g. *Mangifera indica*), banana (*Musa*, e.g. *Musa acuminata*), berries (such as currant, *Ribes*, e.g. *rubrum*), kiwifruit (*Actinidia*, e.g. *chinensis*), grapes (*Vitis*, e.g. *vinifera*), bell peppers (*Capsicum*, e.g. *Capsicum annuum*), cherries (such as the sweet cherry, *Prunus*, e.g. *avium*), cucumber (*Cucumis*, e.g. *sativus*), melons (*Cucumis*, e.g. *melo*), nuts (such as walnut, *Juglans*, e.g.

10 *regia*; peanut, *Arachis hypogaeae*), orange (*Citrus*, e.g. *maxima*), peach (*Prunus*, e.g. *Prunus persica*), pear (*Pyrus*, e.g. *communis*), plum (*Prunus*, e.g. *domestica*), strawberry (*Fragaria*, e.g. *moschata* or *vesca*), tomato (*Lycopersicon*, e.g. *esculentum*); leaves and forage, such as alfalfa (*Medicago*, e.g. *sativa* or *truncatula*), cabbage (e.g. *Brassica oleracea*), endive (*Cichorium*, e.g. *endivia*), leek (*Allium*, e.g. *porrum*), lettuce (*Lactuca*, e.g. *sativa*), spinach

15 (*Spinacia*, e.g. *oleraceae*), tobacco (*Nicotiana*, e.g. *tabacum*); roots, such as arrowroot (*Maranta*, e.g. *arundinacea*), beet (*Beta*, e.g. *vulgaris*), carrot (*Daucus*, e.g. *carota*), cassava (*Manihot*, e.g. *esculenta*), turnip (*Brassica*, e.g. *rapa*), radish (*Raphanus*, e.g. *sativus*), yam (*Dioscorea*, e.g. *esculenta*), sweet potato (*Ipomoea batatas*); seeds, including oilseeds, such as beans (*Phaseolus*, e.g. *vulgaris*), pea (*Pisum*, e.g. *sativum*), soybean

20 (*Glycine*, e.g. *max*), cowpea (*Vigna unguiculata*), mothbean (*Vigna aconitifolia*), wheat (*Triticum*, e.g. *aestivum*), sorghum (*Sorghum* e.g. *bicolor*), barley (*Hordeum*, e.g. *vulgare*), corn (*Zea*, e.g. *mays*), rice (*Oryza*, e.g. *sativa*), rapeseed (*Brassica napus*), millet (*Panicum* sp.), sunflower (*Helianthus annuus*), oats (*Avena sativa*), chickpea (*Cicer*, e.g. *arietinum*); tubers, such as kohlrabi (*Brassica*, e.g. *oleraceae*), potato (*Solanum*, e.g. *tuberosum*) and

25 the like; fiber and wood plants, such as flax (*Linum*, e.g. *Linum usitatissimum*), cotton (*Gossypium* e.g. *hirsutum*), pine (*Pinus* spp.), oak (*Quercus* sp.), eucalyptus (*Eucalyptus* sp.), and the like; and ornamental plants such as turfgrass (*Lolium*, e.g. *rigidum*), petunia (*Petunia*, e.g. *x hybrida*), hyacinth (*Hyacinthus orientalis*), carnation (*Dianthus* e.g. *caryophyllus*), delphinium (*Delphinium*, e.g. *ajacis*), Job's tears (*Coix lacryma-jobi*),

30 snapdragon (*Antirrhinum majus*), poppy (*Papaver*, e.g. *nudicaule*), lilac (*Syringa*, e.g. *vulgaris*), hydrangea (*Hydrangea* e.g. *macrophylla*), roses (including *Gallicas*, *Albas*,

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*Damasks, Damask Perpetuals, Centifolias, Chinas, Teas and Hybrid Teas*) and ornamental goldenrods (e.g. *Solidago spp.*).

Given the conservation of basic metabolic pathways among all eukaryotes, cell collections of the present invention can also usefully be drawn from lower eukaryotes, such as yeasts, particularly *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Pichia* species, such as *methanolica*, *Ustilago maydis*, and *Candida* species, from roundworms, such as *C. elegans*, from zebra fish, and from *Drosophila melanogaster*.

Eukaryotic cell lines from which coisogenic collections of the present invention may be created are readily available from a wide variety of sources known in the art, including the American Type Culture Collection (Manassas, VA, USA), the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, German Collection of Microorganisms and Cell Cultures), and the Riken Cell bank of Japan; 472 such culture collections are listed at <http://wdcm.nig.ac.jp/hpcc.html>.

Specialized cell collections are also well known, and include the NIGMS (National Institute of General Medical Studies) Human Genetic Cell Repository, the NIA Aging Cell Repository, the Autism Research Resource, the ADA Cell Repository Maturity Onset Diabetes Collection, and the HBDI Cell Repository Juvenile Diabetes Collection, all of which are maintained at the Coriell Institute for Medical Studies (Camden, NJ, USA). Specialized yeast collections include the National Collection of Yeast Cultures (Institute of Food Research, Norwich Research Park, Colney, Norwich, UK).

Existing cell lines are also amply well described in the literature. See, e.g., Drexler, The Leukemia-Lymphoma Cell Line FactsBook, (ISBN: 0122219708) (2000); Hay *et al.* (eds.), Atlas of Human Tumor Cell Lines, Academic Press, 1994 (ISBN: 0123335302); Masters *et al.* (eds.), Human Cell Culture: Cancer Cell Lines :Leukemias and Lymphomas, Vol. 3, Kluwer Academic, 2000 (ISBN: 079236225X); Dix (ed.), Plant Cell Line Selection: Procedures and Applications, John Wiley and Sons, 1990 (ISBN:3527279636); Panchal (ed.), Yeast Strain Selection, Marcel Dekker, 1990 (ISBN: 0824782763).

Furthermore, methods are well known in the art for creating immortalized cell lines from a wide variety of primary cells having advantageous characteristics. For recent reviews see, e.g., Yeager *et al.*, "Constructing immortalized human cell lines," *Curr. Opin. Biotechnol.* 10(5):465-9 (1999); Rhim, "Development of human cell lines from multiple

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organs," *Ann. NY Acad. Sci.* 919:16-25 (2000); McLean, "Improved techniques for immortalizing animal cells," *Trends Biotechnol.* 11(6):232-8 (1993); and Hopfer *et al.*, "Immortalization of epithelial cells," *Am. J. Physiol.* 270(1 Pt 1):C1-C11 (1996).

Although at times preferred for convenience, the genotypically distinct cells  
5 need not be immortalized, or otherwise capable of indefinite propagation.

The collection includes at least 5 coisogenic cells (typically, as clonal cell lines). Higher assay throughput is often obtained when the collection includes greater than 5, such as 6, 7, 8, 9, or 10 genotypically distinct, coisogenic cells. Collections of 24 coisogenic cells can conveniently be disposed in a 24 well culture plate; collections of 96  
10 coisogenic cells can conveniently be arrayed in a 96 well microtiter dish. With recent development of microtiter dishes with footprint identical to that of the standard microtiter dish, but with higher well density, collections of 384, 864, 1536, 3456, 6144, and as many as 9600 coisogenic cells can readily and usefully be present in the cell collections of the present invention. The collections need not necessarily contain such even numbers of  
15 genotypically distinct exceptionally coisogenic cells, and can thus include any number of genotypically distinct coisogenic cells greater than or equal to 5, including 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500 or more.

At least five of the genotypically distinct cells of the collections of the  
20 present invention are coisogenic at a common, predetermined, target locus. The target locus can be any protein-encoding locus of the cell. As will be further described below, preferred targets for pharmacogenomic studies encode proteins known to be involved in drug resistance and/or drug metabolism.

As defined herein, coisogenic cells have genomic sequence differences at  
25 the target locus that are sufficient to occasion change of at least one amino acid at the target locus. The genotypically distinct cells of the collection are coisogenic to the others of the genotypically distinct cells of the collection.

The methods and compositions for creating the coisogenic cells, which are further described below, readily permit the legacy-free substitution, addition, or deletion of  
30 as few as 1 and as many as 3 consecutive nucleotides in the genomic DNA of the target locus.

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Alterations can include, for example, substitutions of one, two or three contiguous nucleotides, thus effecting a change in the amino acid encoded by one codon or by two adjacent codons. Since the standard genetic code is well known, the nucleotide changes required to effect change from any given codon to one that encodes any other  
5 desired amino acid would be apparent to the skilled artisan; examples are also presented herein below.

In one such embodiment, one predetermined amino acid residue is commonly targeted for change in each of the coisogenic cells; with a minimum of 20 genotypically distinct cells in the collection, each of the commonly occurring natural amino  
10 acids can be present in the collection at the target residue. Residues that are particularly informative as targets are those that occur in the protein at locations of known structural and/or functional importance, such as within highly structured, ligand-binding domains.

In an alternative embodiment, the genotypically distinct cells can differ not at the identical residue, but at successive amino acids of the target protein. By way of  
15 example, each genotypically distinct cell can contain a single alanine substitution. Thus, without disturbing the initiator methionine, the first cell of the collection can have alanine substituted for residue 2; the second cell of the collection can have alanine substituted for residue 3; the third cell of the collection can have alanine substituted for residue 4, *etc.* Collectively, the coisogenic cells of the cell collection present an *in vivo* alanine scan of the  
20 entire protein sequence, permitting ready identification of critical residues of the target protein.

Any amino acid can be used as the substitute in such an embodiment, with the choice dictated by the known chemical and biological properties of the naturally occurring amino acids. For example, proline can be substituted to effect disruption of  
25 secondary structures, such as beta sheets or alpha helices; tyrosine can be substituted to provide substrates for tyrosine-kinase mediated post-translational modification; glutamic acid can be substituted to increase local charge density.

Alterations can also include introduction of a termination codon. Because any codon of the target locus can be targeted, coisogenic cells can be collected that each  
30 individually possess a single engineered termination codon, but that collectively present consecutive, single amino acid truncations from the carboxy terminus of the target protein.

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Alterations can also include insertion of an amino acid, through targeted insertion of a novel codon between two existing codons.

Alterations can, in other embodiments, include frameshift mutations, caused by insertion or deletion of 1 or 2 nucleotides. Frameshift can lead to truncation or  
5 elongation, depending upon presence of termination codons in the new reading frame. Introduction of compensating frameshifts (e.g., insertion of a single nucleotide followed, at some distance downstream, by deletion of a single nucleotide), can lead to alteration of a series of amino acids between the mutated nucleotides.

Other types of changes that can be created by targeted point mutations will  
10 be readily apparent to one skilled in the art.

Among the changes that can usefully be made, and that have particular utility for pharmacogenomic studies, are those that recapitulate naturally- occurring allelic variants at the target locus; such changes permit the phenotype occasioned by a naturally occurring alleles to be assessed against a common, defined, genetic background.

15 As would be understood, highly multiplex analyses can be done by combining the mutational embodiments set forth above. For example, the collection can include cells that are coisogenic at a first residue of the target locus, with the collection including all possible amino acids at that first target residue, with the collection further including cells that have substitutions at other residues of the target locus.

20 Greater differences can be achieved by targeting changes iteratively to the target locus using the methods of the present invention.

Furthermore, changes can be introduced into both alleles of the target locus, either in a single step or by iterative modification, thus creating a homozygous change. At present, homozygous changes are most desired, although heterozygous  
25 changes are permitted.

In certain embodiments, the coisogenic cells are legacy-free.

In certain embodiments, our methods for constructing coisogenic cell collections, further described below, can alter genomic DNA without concomitant insertion of heterologous nucleic acids, such as selectable markers, prokaryotic genetic elements,  
30 bacteriophage genetic elements, or eukaryotic viral elements, at the target locus. Because such heterologous nucleic acid close to the target locus can cause unpredictable changes in



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expression and/or activity of the target protein, they are disfavored, although permitted, in certain embodiments of the cell collections of the present invention.

Depending on their distance from a common cellular ancestor, the coisogenic cells of the present invention will, on occasion, have accumulated genetic differences at other than the target locus. Such differences are permissible.

In certain particularly useful embodiments, however, the coisogenic cells of the collections of the present invention, are "exceptionally coisogenic", differing in genomic sequence by no more than 0.05%, excluding changes at the target locus. In other embodiments, the cells are "perfectly coisogenic", differing in genomic sequence by no more than about 0.005%, excluding changes at the target locus. The exceptionally coisogenic cell collections and perfectly coisogenic cell collections of the present invention can each, additionally, be legacy-free.

The coisogenic cells of the cell collections of the present invention can also include intentional genetic changes at locations in the genome other than the target locus. For example, mutations can be targeted to a second target locus, creating cell lines that are coisogenic at several targets.

As another example, markers, including selectable markers, can usefully, but optionally, be included, at a site other than the target locus. Such marker can be common to all cells in the collection, for example by prior introduction into a cellular ancestor common to all of the genotypically distinct cells, can be unique to each genotype, or can be common to some, but not to all, genotypically distinct cells in the collection.

For example, a selectable marker can commonly be included in all of the genotypically distinct cells of the collection to prevent overgrowth, either by cells of the same lineage, or by other species. Selectable markers are well known, and the choice thereof will depend upon the species from which the genotypically distinct cells of the collection are derived. Selectable markers for use in mammalian cells, e.g., include markers that confer resistance to neomycin (G418), blasticidin, hygromycin or to zeocin; other well-known selections are based upon the purine salvage pathway. Selectable markers in yeast include a variety of auxotrophic markers, such as alleles of URA3, HIS3, LEU2, TRP1 and LYS2.

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At the other end of the spectrum, unique markers can be introduced into each of the genotypically distinct cells of the collection, allowing each genotypically distinct cell (typically, cell line) in the collection readily to be distinguished.

For example, the sequence can encode substrate-independent  
5 proteinaceous fluorophores with distinct emission spectra. See, e.g., Palm *et al.*, "Spectral Variants of Green Fluorescent Protein," in Green Fluorescent Proteins, Conn (ed.), *Methods Enzymol.* vol. 302, pp. 378 - 394 (1999)), the disclosure of which is incorporated herein by reference.

The markers can also be intended to distinguish the cells at the nucleic  
10 acid, rather than protein, level (genetic "bar codes"). If such bar codes are flanked by priming sites that are common to all of the bar codes of distinct sequence, a single amplification reaction (e.g., by PCR), can be used to stoichiometrically to amplify all bar codes, the presence and/or frequencies of which can thereafter readily be assayed. See, e.g., U.S. Patent No. 6,046,002.

15 Other genetic alterations that can usefully be made outside the target locus include those that facilitate assay of the cells of the coisogenic cell collection of the present invention, as will be discussed below.

The target locus for the coisogenic cell collections of the present invention can be any locus believed to contribute to a relevant cellular or organismic phenotype, and  
20 thus usefully includes all proteins that are presently subject to drug screening assays (e.g., G protein coupled receptors, protein kinases, zinc finger-containing transcription factors), or pharmacogenomic analysis (such as ApoE, presenilin 1, presenilin 2, p53, etc.). Particularly useful targets in certain embodiments of the present invention are loci that encode proteins that affect drug responsiveness, in part because the clinical phenotype can readily be  
25 correlated with a cellular phenotype, permitting ready assay *in vitro*.

Accordingly, the cell collections of the present invention can usefully be coisogenic at loci that encode any one of the P450 enzymes, which are known significantly to affect the metabolism of many, if not most, therapeutic agents.

The cytochrome P450 superfamily includes a large number (as many as 60  
30 in human beings) of separate, but related, monooxygenases that play a central role in oxidative metabolism of a wide range of compounds, including therapeutic drugs. Although

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the number of known P450 enzymes is large, and the endogenous substrates of most unknown, a half dozen or so appear to be responsible for metabolism of the vast majority of prescribed and over-the-counter drugs: **CYP1A2**, **CYP2C17**, **CYP2D6**, **CYP2E** ("**CYP2E1**"), **CYP3A4**, and **CYP4A11**. For recent reviews, see Anzenbacher *et al.*, "Cytochromes P450 and metabolism of xenobiotics," *Cell. Mol. Life Sci.* 58(5-6):737-47 (2001), and *Drug. Ther. Bull.* 38(12):93-5 (2000).

The cell collections of the present invention can thus usefully be coisogenic at **CYP1A2** (cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide 2) (also known as CP12, P3-450, P450(PA)). This gene, the human homologue of which is located about 25 kb away from CYP1A1 on chromosome 15 (at 15q22-qter), encodes a member of the cytochrome P450 superfamily of enzymes closely related to CYP1A1. The gene is aromatic compound-inducible, and is known to metabolize acetaminophen in human beings to the cytotoxic metabolite N-acetylbenzoquinoneimine (NABQI), Thatcher *et al.*, *Cancer Gene Ther.* 7(4):521-5 (2000).

**CYP2C17** can also usefully be targeted.

**CYP2D6** (also known as CPD6, CYP2D, CYP2D@, P450C2D, P450-DB1) encodes cytochrome P450, subfamily IID (debrisoquine, sparteine, etc., -metabolizing), polypeptide 6, and is known to metabolize as many as 20% of commonly prescribed drugs; the cell collections of the present invention can usefully be coisogenic at this locus.

The enzyme's substrates include debrisoquine, an adrenergic-blocking drug; sparteine and propafenone, both anti-arrhythmic drugs; and amitriptyline, an anti-depressant. The gene is highly polymorphic in the population; certain alleles result in the poor metabolizer phenotype, characterized by a decreased ability to metabolize the enzyme's substrates. The gene is located near two cytochrome P450 pseudogenes on chromosome 22q13.1.

**CYP2E** (earlier denominated CPE1, CYP2E1, P450-J, P450C2E) encodes cytochrome P450, subfamily IIE (ethanol-inducible), located in the human genome at 10q24.3-qter, and can usefully be targeted in constructing coisogenic cell collections of the present invention. This P450 enzyme localizes to the endoplasmic reticulum and is induced by ethanol, the diabetic state, and starvation. The enzyme metabolizes both endogenous substrates, such as ethanol, acetone, and acetal, as well as exogenous substrates including

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benzene, carbon tetrachloride, ethylene glycol, and nitrosamines which are premutagens found in cigarette smoke. Due to its many substrates, this enzyme may be involved in such varied processes as gluconeogenesis, hepatic cirrhosis, diabetes, and cancer.

Another locus at which the cell collections of the present invention can  
5 usefully be coisogenic is **CYP3A4** (also known as CP34, NF-25, P450C3, P450PCN1), which encodes cytochrome P450, subfamily IIIA (nifedipine oxidase), polypeptide 4.

The enzyme encoded by CYP3A4 localizes to the endoplasmic reticulum and its expression is induced by glucocorticoids and some pharmacological agents. This enzyme is involved in the metabolism of approximately half the drugs used today, including  
10 nifedipine, acetaminophen, codeine, cyclosporin A, diazepam and erythromycin. The enzyme also metabolizes some steroids and carcinogens.

Vinca alkaloids are important chemotherapeutic agents, and their pharmacokinetic properties display significant interindividual variations, possibly due to CYP3A4-mediated metabolism. See, Yao *et al.*, "Detoxication of vinca alkaloids by human  
15 P450 CYP3A4-mediated metabolism: implications for the development of drug resistance," *J. Pharmacol. Exp. Ther.* 294(1):387-95 (2000).

This gene is part of a cluster of cytochrome P450 genes on chromosome 7q21.1. Previously, another CYP3A gene, CYP3A3, was thought to exist; however, it is now thought that this sequence represents a transcript variant of CYP3A4.

20 **CYP4A11** (also called CP4Y, CYP4A2, CYP4AII), encodes cytochrome P450, subfamily IVA, polypeptide 11, and can usefully serve as a target locus for the coisogenic cell collections of the present invention. CYP4A11 encodes a member of the cytochrome P450 superfamily of enzymes. This protein localizes to the endoplasmic reticulum and hydroxylates medium-chain fatty acids such as laurate and myristate.

25 Other cytochrome P450 enzymes can also usefully be targeted.

**CYP1B1** (synonyms: CP1B, GLC3A), another target at which the cell collections of the present invention can usefully be coisogenic, encodes cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile), located in the human genome at 2p21. The P450 monooxygenase encoded by this gene localizes to the  
30 endoplasmic reticulum and metabolizes procarcinogens such as polycyclic aromatic hydrocarbons and 17beta-estradiol. Mutations in this gene have been associated with

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primary congenital glaucoma; therefore it is thought that the enzyme also metabolizes a signaling molecule involved in eye development, possibly a steroid.

Expression of CYP1B1, as with expression of CYP1A1, has been shown to be increased in an anti-estrogen-resistant breast cell line, Brockdorff *et al.*, *Int. J. Cancer* 88(6):902-6 (2000), and has been generally implicated in tumor drug resistance, Rochat *et al.*, "Human CYP1B1 and anticancer agent metabolism: mechanism for tumor-specific drug inactivation?", *J. Pharmacol. Exp. Ther.* 296(2):537-41 (2001); McFadyen *et al.*, "Cytochrome P450 CYP1B1 protein expression: a novel mechanism of anticancer drug resistance," *Biochem Pharmacol.* 62(2):207-12 (2001).

10           **CYP1A1** (cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide1) (also known as AHH, AHRR, CP11, CYP1, P1-450, P450-C, P450DX), the human homologue of which is located at 15q22-24, can also usefully be targeted. Expression and activity of CYP1A are known to be induced by some polycyclic aromatic hydrocarbons (PAHs), some of which are found in cigarette smoke, and the enzyme is able  
15           to metabolize some PAHs to carcinogenic intermediates; the gene has specifically been associated with lung cancer risk.

CYP1A activity has been shown to be increased in a breast cell line resistant to the antiestrogen compound ICI 182780I, Brockdorff *et al.*, "Increased expression of cytochrome p450 1A1 and 1B1 genes in anti-estrogen-resistant human breast cancer cell  
20           lines," *Int. J. Cancer* 88(6):902-6 (2000), and has been suggested as a marker for sensitivity to anti-cancer drugs, Peters *et al.*, "A mutation in exon 7 of the human cytochrome P-4501A1 gene as marker for sensitivity to anti-cancer drugs?", *Br. J. Cancer* 75(9):1397 (1997).

Another target for which cell collections of the present invention can  
25           usefully be coisogenic is **CYP2A6**, the human homologue of which is found at 19q13.2, encoding cytochrome P450, subfamily IIA (phenobarbital-inducible), polypeptide 6 (also known as CPA6, CYP2A3). CYP2A6 encodes a P450 enzyme that localizes to the endoplasmic reticulum; its expression is induced by phenobarbital. The enzyme is known to hydroxylate coumarin, and also metabolizes nicotine, aflatoxin B1, nitrosamines, and some  
30           pharmaceuticals.

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Individuals with certain allelic variants of CYP2A6 are said to have a "poor metabolizer" phenotype, meaning they do not efficiently metabolize drugs that are substantially metabolized by CYP2A6, such as coumarin, nicotine, or fluoxetine (Prozac®). CYP2A6 is part of a large cluster of cytochrome P450 genes from the CYP2A, CYP2B and  
5 CYP2F subfamilies on chromosome 19q.

CYP2A6 is predominantly responsible for the metabolism of nicotine to cotinine, and many allelic variants have been described. See, Zabetian *et al.*, "Functional variants at CYP2A6: new genotyping methods, population genetics, and relevance to studies of tobacco dependence," *Am. J. Med. Genet.* 96(5):638-45 (2000).

10 Another cytochrome P450 enzyme that can usefully be targeted in the coisogenic cell collections of the present invention is **CYP2A13** (also known as CPAD), the human homologue of which is located at 19q13.2. CYP2A13 is phenobarbital-inducible, and is highly active in the metabolic activation of a major tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, with a catalytic efficiency much greater than  
15 that of other human cytochrome P450 isoforms. Su *et al.*, "Human cytochrome P450 CYP2A13: predominant expression in the respiratory tract and its high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone," *Cancer Res* 60(18):5074-9 (2000).

**CYP2B6** (alternatively denominated CPB6, IIB1, P450, and CYP2B6),  
20 encoding cytochrome P450, subfamily IIA (phenobarbital-inducible), polypeptide 6, is located at 19q13.2 in the human genome, and is a useful target locus for the coisogenic cell collections of the present invention. This P450 enzyme localizes to the endoplasmic reticulum and its expression is induced by phenobarbital. The enzyme is known to metabolize some xenobiotics, such as the anti-cancer drugs cyclophosphamide and  
25 ifosphamide. Transcript variants for this gene have been described; however, it has not been resolved whether these transcripts are in fact produced by this gene or by a closely related pseudogene, CYP2B7. Both the gene and the pseudogene are located in the middle of a CYP2A pseudogene found in a large cluster of cytochrome P450 genes from the CYP2A, CYP2B and CYP2F subfamilies on chromosome 19q. CYP2B6 is thought to  
30 mediate the N-demethylation of (R)- and (S)-ketamine in human liver.

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**CYP2C8** (same as CPC8, P450 MP-12/MP-20) encoding cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 8, is also a useful target for the coisogenic eukaryotic cell collections of the present invention. This protein localizes to the endoplasmic reticulum and its expression is induced by phenobarbital. The enzyme is known to metabolize many xenobiotics, including the anticonvulsive drug mephenytoin, benzo(a)pyrene, 7-ethoxycoumarin, and the anti-cancer drug paclitaxel (Taxol®). CYP2C8 also metabolizes cerivastatin, which is a high potency, third generation synthetic statin with proven lipid-lowering efficacy.

Two transcript variants for this gene have been described; it is thought that the longer form does not encode an active cytochrome P450 since its protein product lacks the heme binding site. This gene is located within a cluster of cytochrome P450 genes on chromosome 10q24.

Another useful target for the coisogenic cell collections of the present invention is **CYP2C9** (cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 9), whose expression is induced by rifampin, and which is known to metabolize many xenobiotics, including phenytoin, tolbutamide, ibuprofen, aspirin and S-warfarin. See, e.g., Bigler *et al.*, "CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk," *Cancer Res.* 61(9):3566-9 (2001).

Studies identifying individuals who are poor metabolizers of phenytoin and tolbutamide suggest that this gene is polymorphic. The gene is located within a cluster of cytochrome P450 genes on chromosome 10q24.

**CYP11A** (same as P450SCC, cytochrome P450C11A1), also usefully targeted in the coisogenic cell collections of the present invention, encodes a member of the cytochrome P450 superfamily of enzymes. This protein localizes to the mitochondrial inner membrane and catalyzes the conversion of cholesterol to pregnenolone, the first and rate-limiting step in the synthesis of the steroid hormones. The human homologue is located at 15q23-q24.

**CYP2C19** (same as CPCJ, CYP2C, P450C2C, P450IIC19, microsomal monooxygenase, xenobiotic monooxygenase, mephenytoin 4'-hydroxylase, flavoprotein-linked monooxygenase), encodes cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 19. This protein localizes to the endoplasmic

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reticulum and is known to metabolize many xenobiotics, including the anticonvulsive drug mephenytoin, omeprazole, diazepam, proganil, and some barbiturates. The enzyme is also responsible for the polymorphic (NAT2\*) acetylation of hydrazine and aromatic amine drugs, such as isoniazid, hydralazine, and sulfasalazine. Polymorphism within this gene is  
5 associated with variable ability to metabolize mephenytoin, known respectively as the poor metabolizer phenotype and extensive metabolizer phenotype. The gene is located within a cluster of cytochrome P450 genes on chromosome 10q24, at 10q24.1-q24.3.

Other cytochrome P450 enzymes that can usefully be targeted to create the coisogenic cell collections of the present invention include CYP2F1, CYP2J2, CYP3A5,  
10 CYP3A7 (catalyzes the prenatal 4-hydroxylation of retinoic acid, playing an important role in protecting the human fetus against retinoic acid-induced embryotoxicity, Chen *et al.*, "Catalysis of the 4-hydroxylation of retinoic acids by cyp3a7 in human fetal hepatic tissues," *Drug. Metab. Dispos.* 28(9):1051-7 (2000)), CYP4B1, CYP4F2 (found to catalyze hydroxylation and dealkylation of an H(1)-antihistamine prodrug, ebastine, Hashizume *et al.*,  
15 "A novel cytochrome p450 enzyme responsible for the metabolism of ebastine in monkey small intestine," *Drug Metab. Dispos.* 29(6):798-805 (2001)), CYP4F3, CYP6D1, CYP6F1 (related to CYP6D1 and involved in pyrethroid detoxification in insects), CYP7A1, CYP8, CYP11A, CYP11B1, CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, and CYP51.

20 Other loci that affect drug resistance are also useful targets for oligonucleotide-mediated alterations for creating eukaryotic coisogenic cell collections of the present invention.

Among such non-P450 loci are the genes encoding ATP-binding cassette (ABC) proteins, which transport various molecules across extra- and intra-cellular  
25 membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White); some members are well known to confer a multi-drug (multiple drug) resistance phenotype on tumor cells.

Best known among the ABC proteins is **ABCB1** (ATP-binding cassette, sub-family B (MDR/TAP), member 1), known alternatively as MDR1 (multi drug resistance  
30 1), P-GP (P-glycoprotein), PGY1, ABC20, and GP170, the human homologue of which maps to 7q21.1.



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The protein encoded by this gene is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multidrug-resistant cells and often mediates the development of resistance to anticancer drugs. A number of studies have demonstrated a negative correlation between Pgp expression levels and chemosensitivity or survival in a range of human malignancies. Lehne, "P-glycoprotein as a drug target in the treatment of multidrug resistant cancer," *Curr. Drug Targets* 1(1):85-99 (2000).

P-glycoprotein is also expressed in normal tissues with excretory function such as liver, kidney and intestine. Apical expression of P-glycoprotein in such tissues results in reduced drug absorption from the gastrointestinal tract and enhanced drug elimination into bile and urine. Moreover, expression of P-glycoprotein in the endothelial cells of the blood-brain barrier prevents entry of certain drugs into the central nervous system. Human P-glycoprotein has been shown to transport a wide range of structurally unrelated drugs such as digoxin, quinidine, cyclosporin and HIV-1 protease inhibitors. Studies in humans indicate a particular importance of intestinal P-glycoprotein for bioavailability of the immunosuppressant cyclosporin. Moreover, induction of intestinal P-glycoprotein by rifampin has now been identified as the major underlying mechanism of reduced digoxin plasma concentrations during concomitant rifampin therapy. For reviews, see Fromm, "P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs," *Int. J. Clin. Pharmacol. Ther.* 38(2):69-74 (2000); Schinkel, "P-Glycoprotein, a gatekeeper in the blood-brain barrier," *Adv. Drug Deliv. Rev.* 36(2-3):179-194 (1999); Van Asperen *et al.*, "The pharmacological role of P-glycoprotein in the intestinal epithelium," *Pharmacol Res.* 37(6):429-35 (1998); Tanigawara, "Role of P-glycoprotein in drug disposition," *Ther. Drug Monit.* 22(1):137-40 (2000); and Schinkel, "The physiological function of drug-transporting P-glycoproteins," *Semin. Cancer Biol.* 8(3):161-70 (1997).

Allelic variants of ABCB1 (MDR1) are known to affect its selectivity and/or activity. Hoffmeyer *et al.*, "Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo," *Proc. Natl. Acad. Sci USA* 97(7):3473-8 (2000); Choi *et al.*,

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"An altered pattern of cross-resistance in multidrug-resistant human cells results from spontaneous mutations in the *mdr1* (P-glycoprotein) gene," *Cell* 53(4):519-29 (1988).

**ABCB4** (ATP-binding cassette, sub-family B (MDR/TAP), member 4)(also known as MDR3, PGY3, ABC21, MDR2/3, PFIC-3) (human homologue maps to 7q21.1), is  
5 another useful target locus for the coisogenic cell collections of the present invention.

The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABCB4 is a member of the MDR/TAP subfamily. Members of the MDR/TAP subfamily are involved in multidrug resistance as well as antigen presentation. This gene encodes a full transporter and  
10 member of the p-glycoprotein family of membrane proteins with phosphatidylcholine as its substrate.

**ABCC1** — ATP-binding cassette, sub-family C (CFTR/MRP), member 1 — (same as MRP, ABCC, GS-X, MRP1, ABC29) is a member of the MRP subfamily of ATP-binding cassette (ABC) proteins, and is involved in multi-drug resistance. This protein  
15 functions as a multispecific organic anion transporter, with oxidized glutathione, cysteinyl leukotrienes, and activated aflatoxin B1 as known substrates. This protein also transports glucuronides and sulfate conjugates of steroid hormones and bile salts. Alternative splicing by exon deletion results in several splice variants but maintains the original open reading frame in all forms.

**ABCC2** (same as DJS, MRP2, cMRP, ABC30, CMOAT, Canalicular multispecific organic anion transporter) encodes ATP-binding cassette, sub-family C (CFTR/MRP), member 2, and is a useful target locus for the coisogenic cell collections of the present invention. ABCC2 is a member of the MRP subfamily of ATP binding cassette proteins, and is involved in multi-drug resistance. This protein is expressed in the  
20 canalicular (apical) part of the hepatocyte and functions in biliary transport. Known substrates include anticancer drugs such as vinblastine.

Another ATP binding cassette protein usefully targeted in the coisogenic cell collections of the present invention is **ABCC3** (also known as MLP2, MRP3, ABC31, CMOAT2, MOAT-D, EST90757), the human homologue of which is located at 17q22. The  
30 protein may play a role in the transport of biliary and intestinal excretion of organic anions. Alternative splicing of this gene results in three known transcript variants.

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Also a useful target for the coisogenic cell collections of the present invention is ATP-binding cassette, sub-family C (CFTR/MRP), member 4, **ABCC4**, also known as MRP4, MOATB, MOAT-B, EST170205. The protein encoded by this gene is a member of the MRP subfamily of ABC transporters, and is involved in multi-drug resistance.

- 5 The protein may play a role in cellular detoxification as a pump for its substrate, organic anions.

- Other useful ABC transporter proteins that can usefully serve as the target locus for the coisogenic cell collections of the present invention include **ABCC4** (MRP4), **ABCC5** (MRP5) (provides resistance to thiopurine anticancer drugs, such as
- 10 6-mercaptopurine and thioguanine, and the anti-HIV drug 9-(2-phosphonylmethoxyethyl)adenine; this protein may be involved in resistance to thiopurines in acute lymphoblastic leukemia and antiretroviral nucleoside analogs in HIV-infected patients); **ABCC6** (MRP6), **MRP7** (CFTR), **ABCC8** (MRP8), **ABCC9**, **ABCC10**, **ABCC11** (same as **HLI**, **SUR**, **MRP8**, **PHH1**, **SUR1**, **ABC36**, **HRINS**), and **ABCC12** (same as
- 15 **MRP9**).

- Other useful targets include **EPHX1** (epoxide hydrolase 1, microsomal xenobiotic), **EPHX2** (epoxide hydrolase 2), **LTA4H** (leukotriene A4 hydrolase), **TRAG3** (**Taxol**<sup>®</sup> resistance associated gene 3, which is overexpressed in most melanoma cells and confers resistance to paclitaxel, **Taxol**<sup>®</sup>), **GUSB** (beta-glucuronidase), **TMPT** (thiopurine
- 20 methyltransferase), **BCRP**, (breast cancer resistance protein, an ATP transporter), dihydropyrimidine dehydrogenase, **HERG** (involved in drug transport through potassium ion channels), **hKCNE2** (involved in drug transport through potassium ion channels), **UDP glucuronosyl transferase (UGT)** (a hepatic metabolizing enzyme, a detoxifying enzyme for most carcinogens after different cytochrome P450 (CYP) isoforms), sulfotransferase,
- 25 sulfatase, and glutathione S-transferase (**GST**) -alpha, -mu, -pi (which detoxify therapeutic drugs, not least several anti-cancer drugs), **ACE** (peptidyl-dipeptidase A), and **KCHN2** (potassium voltage-gated channel, subfamily H (eag-related), member 2), location 7q35-q36).

- Another protein usefully targeted in the coisogenic cell collections of the
- 30 present invention is the **BCR-ABL** fusion responsible for chronic myeloid leukemia. The tyrosine kinase domain of the fusion protein is targeted by imatinib (**Gleevec**); allelic

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variants have been identified that confer polyclonal resistance to the drug. Shah *et al.*, *Cancer Cell* 2:117-125 (2002), incorporated herein by reference in its entirety.

Another protein usefully targeted in the coisogenic cell collections of the present invention is beta tubulin. Paclitaxel is a tubulin-disrupting agent that binds  
5 preferentially to beta-tubulin. Allelic variants of beta tubulin have been identified that confer resistance to paclitaxel. Giannakakou *et al.*, *J. Biol. Chem.* 272:17118-17125 (1997), incorporated herein by reference in its entirety.

As noted above, the coisogenic cell collections of the present invention can usefully include  
10 cells that have, at the coisogenic target locus, the sequence of a naturally-occurring allele; this permits the phenotype conferred by the allele to be assessed without the confounding presence of other genetic differences at the target locus or elsewhere in the cellular genome. Accordingly, the coisogenic cell collections of the present invention can usefully include cells that have the naturally occurring (allelic) variants set forth in the following  
15 tables.

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Table 1

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>ABCB1</b> "ATP-binding cassette, sub- family B (MDR/TAP), member 1"	7q21.1	X58723 and X59732	4643 bp 1279 aa	
10	(MDR1, P-GP, PGY1, ABC20, GP170, P- Glycoprotein)		AC002457, AC005068 (g) AF016535, M14758 (m) NP_000918 (p)		

## Allelic Variants from Scientific Literature

	DNA Variant	Protein Variant	Phenotype	References
15	GGA > GTA	Gly185Val	Correlated with increased colchicine resistance	OMIM 171050, Safa <i>et al.</i> (1990), Choi <i>et al.</i> (1988)
	G2995A GCT > TCT, G2677T	Ala999Thr Ala893Ser	Unknown "correlations of mutations with expression levels"	Mickley <i>et al.</i> (1998) Tanabe <i>et al.</i> (2001), Cascorbi <i>et al.</i> (2001)
20	GCT > ACT, G2677A	Ala893Thr	"correlations of mutations with expression levels"	Tanabe <i>et al.</i> (2001), Cascorbi <i>et al.</i> (2001)
	AAT > GAT, A61G	Asn21Asp	Unknown	Cascorbi <i>et al.</i> (2001), Hoffmeyer <i>et al.</i> (2000), WO 01/09183
25	AGT > AAT, G1199A	Ser400Asn	"may correlate with low expression" WO 01/09183 (p40)	Cascorbi <i>et al.</i> (2001), Hoffmeyer <i>et al.</i> (2000), WO 01/09183
	CAG > CCG, A3320C	Gln1107Pro	Unknown	Cascorbi <i>et al.</i> (2001)
	CAG > ???, A3320?	Phe103Ser	Unknown	WO 01/09183 (p7)
30	TTC > CTC, T307C	Phe103Leu	Unknown	Hoffmeyer <i>et al.</i> (2000), WO 01/09183
	ATC > ATT, C3435T	Ile1145Ile (wobble)	Correlated with (2X) lower p- glycoprotein expression and activity	OMIM 171050, Hoffmeyer <i>et al.</i> (2000)

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**Allelic Variants from SNP Database**

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_017168	4730224	rs2235039	XP_029059	g	V	1	801
					a	M		
5	NT_017168	4735268	rs2032581	XP_029059	a	I	1	829
					g	V		

**Table 2**

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
10	<b>ABCB4</b> "ATP-binding cassette, sub- family B (MDR/TAP), member 4"	7q21.1	NT_017168 (working draft chromo7) M23234,Z35284 (m)	5764, 5785, I and 5623 bp 1279, 1286, and 1232 aa	
15	(MDR3, PGY3, ABC21, MDR2/3, PFIC- 3, P- glycoprotein 3)				

20

**Allelic Variants from Scientific Literature:**

DNA Variant	Protein Variant	Phenotype	References
CGA > TGA	Arg957Ter	Cholestasis	OMIM 171060

**Allelic Variants from SNP Database:**

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
25	NT_017168	4860286	rs31655	XP_004599	g	A	1	1107
					a	T		

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Table 3

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>ABCC1</b> "ATP-binding cassette, sub- family C (CFTR/MRP), member 1"	16p13.1	AF022824 (exon2) AF022825 (exon3) AF022826 (exon4) AF022827 (exon5) AF022828 (exon6) AF022829 (exon7) AF022830 (exon8) AF022831 (exon9) AF022832 (exon10) AF017145 (5'flanking sequence)	5927, 5749, and 5759 bp 1531, 1472, and 1475 aa	
10	(MRP, ABCC, GS-X, MRP1, ABC29)		L05628,U91318 (m) NP_004987 isoform 1 (p) NP_063915 isoform 2 (p) NP_063953 isoform 3 (p)		

## Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
15	G128C	Cys43Ser	Unknown	Ito <i>et al.</i> (2001)
	C218T	Thr73Ile	Unknown	Ito <i>et al.</i> (2001)
	G2168A	Arg723Gln	Unknown	Ito <i>et al.</i> (2001)
	G3173A	Arg1058Gln	Unknown	Ito <i>et al.</i> (2001)

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Table 4

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>ABCC2</b> "ATP-binding cassette, sub- family C (CFTR/MRP), member 2"	10q24	NT_029377 (working draft chromo10) U63970 (m) NP_000383 (p)	4868 bp 1545 aa	
10	(DJS, MRP2, cMRP, ABC30, CMOAT)				

## Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
15	C2302T	Arg768Tr p	Dubin-Johnson syndrome	OMIM 601107, Toh <i>et al.</i> (1999), Wada <i>et al.</i> (1998), Ito <i>et al.</i> (2001)
	A4145G	Gln1382A rg	Dubin-Johnson syndrome	OMIM 601107, Toh <i>et al.</i> (1999).
	G1249A	Val417Ile	Unknown	Ito <i>et al.</i> (2001)
	C2366T	Ser789Ph e	Unknown	Ito <i>et al.</i> (2001)
	G4348A	Ala1450T hr	Unknown	Ito <i>et al.</i> (2001)



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Table 5

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information			
5	<b>ABCC3</b> "ATP-binding cassette, sub- family C (CFTR/MRP), member 3"	17q22	NT_010783 (working draft chromo17)	5176, 5325, and 5380 bp 1527, 1238, and 510 aa				
10	(MLP2, MRP3, ABC31, CMOAT2, MOAT-D, EST90757)		AF009670 (m) AF085690 (m) AF085691 (m) AF085692 (m) NP_003777 (p) NP_064421 (p) NP_064422 (p)					
15	<b>Allelic Variants from SNP Database:</b>							
	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_010783	1635643	rs1051625	XP_008422	c g	L V	1	120
	NT_010783	1619267	_	XP_037992	c g	T R	2	527
20	NT_010783	1619270	rs1003355	XP_037992	c g	A G	2	528
	NT_010783	1629592	rs967935	XP_037992	c t	S F	2	1221
	NT_010783	1619267	rs1003354	XP_037994	c g	T R	2	527
	NT_010783	1619270	rs1003355	XP_037994	c g	A G	2	528
	NT_010783	1635643	rs1051625	XP_037994	c g	L V	1	1362
25	NT_010783	1619267	rs1003354	XP_037997	c g	T R	2	454
	NT_010783	1619270	rs1003355	XP_037997	c g	A G	2	455
	NT_010783	1635643	rs1051625	XP_037997	c g	L V	1	1289
	NT_010783	1619267	rs1003354	XP_037999	c g	T R	2	527
	NT_010783	1619270	rs1003355	XP_037999	c g	A G	2	528
30	NT_010783	1635643	rs1051625	XP_037999	c g	L V	1	1362
	NT_010783	1619267	rs1003354	XP_038002	c g	T R	2	527
	NT_010783	1619270	rs1003355	XP_038002	c	A	2	528

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NT_010783	1635643	rs1051625	XP_038002	g c g	G L V	1	1362
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Table 6

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>ABCC5</b>	3q27	NT_022676 (working draft chromo3)	5838 bp 1437 aa	
10	"ATP-binding cassette, sub- family C (CFTR/MRP), member 5"		NP_005679 (m)		
15	(MRP5, SMRP, ABC33, MOATC, MOAT-C, pABC11, EST277145)		NP_005679 (p)		

## Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
20	NT_022676	100964	rs1053351	XP_002914	c a	Y *	3	1202
	NT_022676	124876	rs1053387	XP_002914	c a	T N	2	1383
25	NT_022676	100964	rs1053351	XP_037577	c a	Y *	3	711
	NT_022676	124876	rs1053387	XP_037577	c a	T N	2	892

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Table 7

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information			
5	<b>ABCC6</b> "ATP-binding cassette, sub- family C (CFTR/MRP), member 6"	16p13.1	U91318 (human BAC clone)	4535 bp 1503 aa				
10	(ARA, PXE, MLP1, MRP6, ABC34, MOATE, EST349056)		AF076622 (m) NP_001162 (p)					
15	<b>Allelic Variants from Scientific Literature:</b>							
	DNA Variant	Protein Variant	Phenotype	References				
	C3421T	Arg1141T er	Pseudoxanthoma Elasticum	OMIM 603234				
	G3413A	Arg1138G In	Pseudoxanthoma Elasticum	OMIM 603234				
	G3341C	Arg1114P ro	Pseudoxanthoma Elasticum	OMIM 603234				
20	C3940T	Arg1314T rp	Pseudoxanthoma Elasticum	OMIM 603234				
	--	Arg1268G In	Pseudoxanthoma Elasticum	OMIM 603234				
	C3412T	Arg1138T rp	Pseudoxanthoma Elasticum	OMIM 603234				
	C3490T	Arg1164T er	Pseudoxanthoma Elasticum	OMIM 603234				
	<b>Allelic Variants from SNP Database:</b>							
25	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_010393	2241302	rs2238472	XP_007798	g a	R Q	2	1268
	NT_010393	2241302	rs2238472	XP_027249	g a	R Q	2	33
30								

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Table 8

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>ABCC8</b>	11p15.1	L78243 (exon39)	4977 bp	
	"ATP-binding cassette, sub- family C (CFTR/MRP), member 8"		U63455 (exon39 and complete cds.)	1581 aa	
10	(HI, SUR, MRP8, PHHI, SUR1, ABC36, HRINS)		NT_009307 (working draft chromo11) AH004854 (m) NP_000343 (p)		

## Allelic Variants from Scientific Literature:

15	DNA Variant	Protein Variant	Phenotype	References
	G > T	Gly716Val	Persistent Hyperinsulinemic Hypoglycemia of Infancy	OMIM 600509
	G4058C	Arg1353P ro	Persistent Hyperinsulinemic Hypoglycemia of Infancy	OMIM 600509
	C4261T	Arg1421C ys	Persistent Hyperinsulinemic Hypoglycemia of Infancy	OMIM 600509
	C4480T	Arg1494T rp	Persistent Hyperinsulinemic Hypoglycemia of Infancy	OMIM 600509

20

## Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_009307	1343092	rs1048098	XP_036346	t	F	1	157
					c	L		
	NT_009307	1343122	rs1048096	XP_036346	c	L	1	167
					g	V		
25	NT_009307	1344909	rs1048095	XP_036346	t	L	2	225
					c	P		
	NT_009307	1345002	rs1048094	XP_036346	c	A	2	256
					t	V		
	NT_009307	1409710	rs757110	XP_036346	g	A	1	1369
					t	S		

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Table 9

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information			
5	<b>ACE</b> "angiotensin I converting enzyme (peptidyl- dipeptidase A) 1" (ACE1, DCP1, CD143)	17q23	NT_010698 (working draft chromo17) J04144 (m) NP_000780 (p)	4020 bp 1306 aa				
10								
Allelic Variants from Scientific Literature:								
15	DNA Variant	Protein Variant	Phenotype	References				
	A2350G	?	"significantly associated with blood pressure"	OMIM 106180				
Allelic Variants from SNP Database:								
	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
20	NT_010698	1458291	rs4348	XP_008260	c t	P L	2	5
	NT_010698	1460620	rs4976	XP_008260	t c	I T	2	94

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Table 10

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP1A1</b> "cytochrome P450, subfamily I (aromatic compound- inducible), polypeptide1" (AHH, AHRR, CP11, CYP1, P1-450, P450- C, P450DX)	15q22-24	X02612 X04300 X02612 (m) X04300 (m) NP_000490 (p)	2602 bp 512 aa	
10					
15					

**Allelic Variants from Scientific Literature:**

DNA Variant	Protein Variant	Phenotype	References
?	Ala462Val	Correlated with increased risk of lung cancer, but may be just marker	OMIM 108330

**Allelic Variants from SNP Database:**

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
20	NT_010374	225016	rs1048943	XP_007727	a g c a c t	I V T N R W	1  2  1	462  461  93

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Table 11

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP1B1</b> "cytochrome P450, subfamily I (dioxin- inducible), polypeptide 1 (glaucoma 3, primary infantile)" (CP1B, GLC3A)	2p21	U56438  X04300 (g) X02612 (g) U56438 (g) U03688 (m) NP_000095 (p)	5128 bp 543aa	
10					
15					

## Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
20	G3976A T3807C G1505A	Trp57Ter Met1Thr Lys387Gl u	Peters Anomaly Peters Anomaly Glaucoma	OMIM 601771 OMIM 601771 OMIM 601771
	G7957A	Asp374As n	Glaucoma	OMIM 601771
	C8242T	Arg469Tr p	Glaucoma	OMIM 601771
	G3987A ?	Gly61Glu Gly365Trp	Glaucoma Glaucoma	OMIM 601771 OMIM 601771

## 25 Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
30	NT_005274	679631	rs10012	XP_002576	c g	R G	1	48
	NT_005274	679844	rs1056827	XP_002576	g t	A S	1	119
	NT_005274	683818	rs1056836	XP_002576	g c	V L	1	432
	NT_005274	683871	rs1056837	XP_002576	t a	D E	3	449
	NT_005274	683882	rs1800440	XP_002576	a g	N S	2	453

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Table 12

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP2A6</b> "cytochrome P450, subfamily IIA (phenobarbital- inducible), polypeptide 6" (CPA6, CYP2A3)	19q13.2	U22027  NM_000762 (m) NP_000753 (p) NG_000008 (g)	1751 bp 494 aa	
10					
	<b>Allelic Variants from Scientific Literature:</b>				
	DNA Variant	Protein Variant	Phenotype		References
15	?	Leu160His	Protein becomes "catalytically inactive"		OMIM 601771

Table 13

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
20	<b>CYP2A7</b> "cytochrome P450, subfamily IIA (phenobarbital- inducible), polypeptide 7" (CPA7, CPAD, CYP11A7, P450-IIA4)	19q13.2	NT_029481 (working draft chromo19)  NG_000008 (g) NM_000764 (m) NP_000755 (p) NP085079 (p)	2281 bp and 2128 bp 494 and 443 aa	
25					
	<b>Allelic Variants from Scientific Literature:</b>				
30	DNA Variant	Protein Variant	Phenotype		References
	T > A	Leu160His	Unknown		OMIM 122720



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Table 14

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP2C8</b> "cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 8" (CPC8, P450 MP- 12/MP-20)	10cen- q26.11	L16876 (exon 9) NT_008769 (working draft 10)  NM_000770 (m) NM_030878 (m) NP_000761 (p) NP_110518 (p)	1851 and 1890 bp 490 and 393 aa	

## Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
15	NT_008769	823719	rs1058930	XP_011938	c	I	3	264
					g	M		
	NT_008769	823719	rs1058930	XP_050924	c	I	3	67
					g	M		
	NT_008769	823719	rs1058930	XP_050926	c	I	3	251
					g	M		
20	NT_008769	823719	rs1058930	XP_050929	c	I	3	264
					g	M		

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Table 15

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP2C9</b> "cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 9"	10q24	NT_008769 (working draft chromo10)	1835 bp 490 aa	
10	(CPC9, CYP2C10, P450IIC9, P450 MP-4, P450 PB-1)		NM_000771 (m) NP_000762 (p)		

## Allelic Variants from Scientific Literature:

DNA Variant	Protein Variant	Phenotype	References
?	Arg144Cys	Warfarin Sensitivity	OMIM 601129
?	Ile359Leu	Poor tolbutamide metabolism (diabetes mellitus)	OMIM 601129

20

## Allelic Variants from SNP Database:

Contig Accession	Contig Position	dbSNPs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
NT_008769	43400	rs1057910	XP_050915	a c	I L	1	21
NT_008769	43402	rs1057909	XP_050915	a g	Y C	2	20

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Table 16

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP2C19</b> "cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 19" (CPCJ, CYP2C, P450C2C, P450IIC19)	10q24.1- q24.3	NT_008769 (working draft chromo10) M61854 (m) NM_000769 (m) NP_000760 (p)	1473 bp 490 aa	arg433-to-trp mutation in the heme-binding region  Ibeanu <i>et al.</i> (1998)
10					
15	<b>Allelic Variants from Scientific Literature:</b>				
	DNA Variant	Protein Variant	Phenotype	References	
	?	Arg433Tr p	Mephenytoin 4- Hydroxylase defect, poor metabolizer	OMIM 124020	

Table 17

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
20	<b>CYP2D6</b> "cytochrome P450, subfamily IID (debrisoquine, sparteine, etc., -metabolizing), polypeptide 6" (CPD6, CYP2D, CYP2D@, P450C2D, P450-DB1)	22q13.1	M33388  NM_000106 (m) NP_000097 (p)	1655 bp 497 aa	
25					
30					
35	<b>Allelic Variants from Scientific Literature:</b>				
	DNA Variant	Protein Variant	Phenotype	References	
	?	Gly169Ter	Debrisoquine, poor drug metabolizer	OMIM 124030	

**Allelic Variants from SNP Database:**

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	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_011520	2165135 9	rs2103556	XP_013013	c	T	2	396
5	NT_011520	2165146 3	rs2070905	XP_013013	g g	S M	3	361
	NT_011520	2165168 6	rs2070907	XP_013013	a a	I K	1	320
	NT_011520	2165224 9	rs1065569	XP_013013	g g	E V	1	284
10	NT_011520	2165227 5	rs1974456	XP_013013	a g	M R	2	275
	NT_011520	2165263 1	rs1800754	XP_013013	a c	H S	2	221
15	NT_011520	2165266 2	rs1058171	XP_013013	t a	L N	1	211
	NT_011520	2165266 4	rs1058170	XP_013013	g g	D G	2	210
	NT_011520	2165306 3	rs1058167	XP_013013	c c	A P	2	141
20	NT_011520	2165135 9	rs2103556	XP_040060	t c	L T	2	140
	NT_011520	2165146 3	rs2070905	XP_040060	g g	S M	3	105
25	NT_011520	2165168 6	rs2070907	XP_040060	a a	I K	1	64
	NT_011520	2165224 9	rs1065569	XP_040060	g g	E V	1	28
	NT_011520	2165227 5	rs1974456	XP_040060	a g	M R	2	19
30	NT_011520	2165135 9	rs2103556	XP_040062	a c	H T	2	140
	NT_011520	2165146 3	rs2070905	XP_040062	g g	S M	3	105
35	NT_011520	2165168 6	rs2070907	XP_040062	a a	I K	1	64
	NT_011520	2165224 9	rs1065569	XP_040062	g g a	E V M	1	28

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	NT_011520	2165227 5	rs1974456	XP_040062	g	R	2	19
	NT_011520	2165135 9	rs2103556	XP_040064	a c	H T	2	180
5	NT_011520	2165146 3	rs2070905	XP_040064	g g	S M	3	145
	NT_011520	2165168 6	rs2070907	XP_040064	a a	I K	1	104
	NT_011520	2165224 9	rs1065569	XP_040064	g g	E V	1	68
10	NT_011520	2165227 5	rs1974456	XP_040064	a g	M R	2	59
	NT_011520	2165263 1	rs1800754	XP_040064	a c	H S	2	5
15	NT_011520	2165135 9	rs2103556	XP_040065	t c	L T	2	227
	NT_011520	2165146 3	rs2070905	XP_040065	g g	S M	3	192
	NT_011520	2165168 6	rs2070907	XP_040065	a a	I K	1	151
20	NT_011520	2165224 9	rs1065569	XP_040065	g g	E V	1	115
	NT_011520	2165227 5	rs1974456	XP_040065	a g	M R	2	106
25	NT_011520	2165263 1	rs1800754	XP_040065	a c	H S	2	52
	NT_011520	2165266 2	rs1058171	XP_040065	t a	L N	1	42
	NT_011520	2165266 4	rs1058170	XP_040065	g g	D G	2	41
30	NT_011520	2165135 9	rs2103556	XP_040066	c c	A T	2	396
	NT_011520	2165146 3	rs2070905	XP_040066	g g	S M	3	361
35	NT_011520	2165168 6	rs2070907	XP_040066	a a	I K	1	320
	NT_011520	2165224 9	rs1065569	XP_040066	g g	E V	1	284

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	NT_011520	2165227 5	rs1974456	XP_040066	a g	M R	2	275
	NT_011520	2165263 1	rs1800754	XP_040066	a c	H S	2	221
5	NT_011520	2165266 2	rs1058171	XP_040066	t a	L N	1	211
	NT_011520	2165266 4	rs1058170	XP_040066	g g	D G	2	210
10	NT_011520	2165305 9	rs1058169	XP_040066	c c	A H	3	142
	NT_011520	2165306 3	rs1058167	XP_040066	t c	H P	2	141
					t	L		

## Allelic variants from Karolinska Institute:

15	Allele	Protein	Nucleotide change s	Trivial name	Effect	Enzyme activity		Reference s
						<i>In vivo</i>	<i>In vitro</i>	
	CYP2D6*1 A	CYP2D6.1	None	Wild-type	.	Normal	Normal	Kimura et al, 1989
	CYP2D6*2 A	CYP2D6.2	-1584CG; - 1235AG; - 740CT; - 678GA; 1661GC; 2850CT; 4180GC	CYP2D6L	R296C; S486T	Normal (dx,d,s)		Johansson et al, 1993 Panerat et al, 1994 Raimundo et al, 2000 See also comment below the table.
20	CYP2D6*2 B	CYP2D6.2	1039CT; 1661GC; 2850CT; 4180GC	.	R296C; S486T	.	.	Marez et al, 1997
	CYP2D6*2 C	CYP2D6.2	1661GC; 2470TC; 2850CT; 4180GC	.	R296C; S486T	.	.	Marez et al, 1997 Sachse et al, 1997
25	CYP2D6*2 D	CYP2D6.2	2850CT; 4180GC	M10	R296C; S486T	.	.	Marez et al, 1997
	CYP2D6*2 E	CYP2D6.2	997CG; 1661GC; 2850CT; 4180GC	M12	R296C; S486T	.	.	Marez et al, 1997
	CYP2D6*2 F	CYP2D6.2	1661GC; 1724CT; 2850CT; 4180GC	M14	R296C; S486T	.	.	Marez et al, 1997

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	CYP2D6*2 CYP2D6.2 G	1661GC; 2470TC; 2575CA; 2850CT; 4180GC	M16	R296C; S486T	.	.	Marez et al, 1997
	CYP2D6*2 CYP2D6.2 H	1661GC; 2480CT; 2850CT; 4180GC	M17	R296C; S486T	.	.	Marez et al, 1997
5	CYP2D6*2 CYP2D6.2 J	1661GC; 2850CT; 2939GA; 4180GC	M18	R296C; S486T	.	.	Marez et al, 1997
	CYP2D6*2 CYP2D6.2 K	1661GC; 2850CT; 4115CT; 4180GC	M21	R296C; S486T	.	.	Marez et al, 1997
10	CYP2D6*2 CYP2D6.2 XN (N=2, 3, 4, 5 or 13)	1661GC; 2850CT; 4180GC	.	R296C; S486T N active genes	Incr (d)	.	Johansson et al, 1993 Dahl et al, 1995 Aklilu et al, 1996
	CYP2D6*3 A	2549Adel	CYP2D6A	Frameshift	None (d, s)	None (b)	Kagimoto et al, 1990
15	CYP2D6*3 B	1749AG; 2549Adel	.	N166D; frameshift	.	.	Marez et al, 1997
	CYP2D6*4 A	100CT; 974CA; 984AG;_9 97CG; 1661GC; 1846GA; 4180GC	CYP2D6B	P34S; L91M; H94R; Splicing defect; S486T	None (d, s)	None (b)	Kagimoto et al, 1990 Gough et al, 1990 Hanioka et al, 1990
20	CYP2D6*4 B	100CT; 974CA; 984AG; 997CG; 1846GA; 4180GC	CYP2D6B	P34S; L91M; H94R; Splicing defect; S486T	None (d, s)	None (b)	Kagimoto et al, 1990
	CYP2D6*4 C	100CT; 1661GC; 1846GA; 3887TC; 4180GC	K29-1	P34S; Splicing defect; L421P; S486T	None	.	Yokota et al, 1993
	CYP2D6*4 D	100CT; 1039CT; 1661GC; 1846GA; 4180GC	.	P34S; Splicing defect; S486T	None (dx)	.	Marez et al, 1997
25	CYP2D6*4 E	100CT; 1661GC; 1846GA; 4180GC	.	P34S; Splicing defect; S486T	.	.	Marez et al, 1997

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	CYP2D6*4 F	100CT; 974CA; 984AG; 997CG; 1661GC; 1846GA; 1858CT; 4180GC	P34S; L91M; H94R; Splicing defect; R173C; S486T		Marez et al, 1997
	CYP2D6*4 G	100CT; 974CA; 984AG; 997CG; 1661GC; 1846GA; 2938CT; 4180GC	P34S; L91M; H94R; Splicing defect; P325L; S486T		Marez et al, 1997
5	CYP2D6*4 H	100CT; 974CA; 984AG; 997CG; 1661GC; 1846GA; 3877GC; 4180GC	P34S; L91M; H94R; Splicing defect; E418Q; S486T		Marez et al, 1997
	CYP2D6*4 J	100CT; 974CA; 984AG; 997CG; 1661GC; 1846GA	P34S; L91M; H94R; Splicing defect		Marez et al, 1997
10	CYP2D6*4 K	100CT; 1661GC; 1846GA; 2850CT; 4180GC	P34S; Splicing defect; R296C; S486T	None	Sachse et al, 1997
	CYP2D6*4 L	100CT; 997CG; 1661GC; 1846GA; 4180GC	P34S; Splicing defect; S486T		Submitted 17-Aug-00 by Dr. T. Shimada
	CYP2D6*4 X2			None	Løvlie et al, 1997 Sachse et al, 1998
15	CYP2D6*5	CYP2D6 deleted	CYP2D6D deleted	CYP2D6 deleted	None (d, s) Gaedigk et al, 1991 Steen et al, 1995
	CYP2D6*6 A	1707Tdel	CYP2D6T	Frameshift	None (d, dx) Saxena et al, 1994
	CYP2D6*6 B	1707Tdel; 1976GA		Frameshift; G212E	None (s, d) Evert et al, 1994 Daly et al, 1995



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	CYP2D6*6 C	.	1707Tdel; 1976GA; 4180GC	.	Frameshift; G212E; S486T	None (s)	.	Marez et al, 1997
	CYP2D6*6 D	.	1707Tdel; 3288GA	.	Frameshift; G373S	.	.	Marez et al, 1997
5	CYP2D6*7 CYP2D6.7		2935AC	CYP2D6E	H324P	None (s)	.	Evert et al, 1994
	CYP2D6*8	.	1661GC; 1758GT; 2850CT; 4180GC	CYP2D6G	Stop codon; R296C; S486T	None (d, s)	.	Broly et al, 1995
	CYP2D6*9 CYP2D6.9		2613- 2615delAG A	CYP2D6C	K281del	Decr (b,s,d)	Decr (b,s,d)	Tyndale et al, 1991 Broly & Meyer, 1993
	CYP2D6*1 0A	CYP2D6.1 0	100CT; 1661GC; 4180GC	CYP2D6J	P34S; S486T	Decr (s)	.	Yokota et al, 1993
10	CYP2D6*1 0B	CYP2D6.1 0	100CT; 1039CT; 1661GC; 4180GC	CYP2D6C h1	P34S; S486T	Decr (d)	Decr (b)	Johansson et al, 1994
	CYP2D6*1 0C			see CYP2D6*36				
15	CYP2D6*1 1	.	883GC; 1661GC; 2850CT; 4180GC	CYP2D6F	Splicing defect; R296C; S486T	None (s)	.	Marez et al, 1995
	CYP2D6*1 2	CYP2D6.1 2	124GA; 1661GC; 2850CT; 4180GC	.	G42R; ; R296C; S486T	None (s)	.	Marez et al, 1996
	CYP2D6*1 3	.	CYP2D7P/ CYP2D6 hybrid. Exon 1 CYP2D7, exons 2-9 CYP2D6.	.	Frameshift	None (dx)	.	Panserat et al, 1995
20	CYP2D6*1 4	CYP2D6.1 4	100CT; 1758GA; 2850CT; 4180GC	.	P34S; G169R; R296C; S486T	None (d)	.	Wang, 1992 Wang et al, 1999
	CYP2D6*1 5	.	138insT	.	Frameshift	None (d, dx)	.	Sachse et al, 1996
25	CYP2D6*1 6	.	CYP2D7P/ CYP2D6 hybrid. Exons1-7 CYP2D7P- related, exons 8-9 CYP2D6.	CYP2D6D 2	Frameshift	None (d)	.	Daly et al, 1996

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	CYP2D6*1 7	CYP2D6.1 7	1023CT; 1638GC; 2850CT; 4180GC	CYP2D6Z	T107I; R296C; S486T	Decr (d)	Decr (b)	Masimirem bwa et al, 1996 Oscarson et al, 1997
	CYP2D6*1 8	CYP2D6.1 8	4125- 4133insGT GCCCCACT	CYP2D6(J 9)	468- 470VPT ins	None (s)	Decr (b)	Yokoi et al, 1996
5	CYP2D6*1 9	.	1661GC; 2539- 2542delAA CT; 2850CT; 4180GC	.	Frameshift; R296C; S486T	None	.	Marez et al, 1997
	CYP2D6*2 0	.	1661GC; 1973insG; 1978CT; 1979TC; 2850CT; 4180GC	.	Frameshift ; L213S; R296C; S486T	None (m)	.	Marez- Allorge et al, 1999
10	CYP2D6*2 1	CYP2D6.2 1	77GA	M1	R26H	.	.	Marez et al, 1997
	CYP2D6*2 2	CYP2D6.2 2	82CT	M2	R28C	.	.	Marez et al, 1997
	CYP2D6*2 3	CYP2D6.2 3	957CT	M3	A85V	.	.	Marez et al, 1997
15	CYP2D6*2 4	CYP2D6.2 4	2853AC	M6	I297L	.	.	Marez et al, 1997
	CYP2D6*2 5	CYP2D6.2 5	3198CG	M7	R343G	.	.	Marez et al, 1997
20	CYP2D6*2 6	CYP2D6.2 6	3277TC	M8	I369T	.	.	Marez et al, 1997
	CYP2D6*2 7	CYP2D6.2 7	3853GA	M9	E410K	.	.	Marez et al, 1997
	CYP2D6*2 8	CYP2D6.2 8	19GA; 1661GC; 1704CG; 2850CT; 4180GC	M11	V7M; Q151E; R296C; S486T	.	.	Marez et al, 1997
25	CYP2D6*2 9	CYP2D6.2 9	1659GA; 1661GC; 2850CT; 3183GA; 4180GC	M13	V136M; R296C; V338M; S486T	.	.	Marez et al, 1997
	CYP2D6*3 0	CYP2D6.3 0	1661GC; 1863 ins 9bp rep; 2850CT; 4180GC	M15	172- 174FRP rep; R296C; S486T	.	.	Marez et al, 1997
30	CYP2D6*3 1	CYP2D6.3 1	1661GC; 2850CT; 4042GA; 4180GC	M20	R296C; R440H; S486T	.	.	Marez et al, 1997

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	CYP2D6*3	CYP2D6.3	1661GC; 2850CT; 3853GA; 4180GC	M19	R296C; E410K; S486T	.	.	Marez et al, 1997
	CYP2D6*3	CYP2D6.3	2483GT	CYP2D6*1 C	A237S	Normal (s)	.	Marez et al, 1997
5	CYP2D6*3	CYP2D6.3	2850CT	CYP2D6*1 D	R296C	.	.	Marez et al, 1997
	CYP2D6*3	CYP2D6.3	31GA; 1661GC; 2850CT; 4180GC	CYP2D6*2 B	V11M; R296C; S486T	Normal (s)	.	Marez et al, 1997
10	CYP2D6*3	CYP2D6.3	31GA; 1661GC; 2850CT; 4180GC	.	V11M; R296C; S486T	Incr	.	Griese et al, 1998
	CYP2D6*3	CYP2D6.3	100CT; 1039CT; 1661GC; 4180GC; gene conversion to CYP2D7 in exon 9	CYP2D6C h2	P34S; S486T	Decr (d)	Decr (b)	Wang, 1992 Johansson et al, 1994 Leathart et al, 1998
	CYP2D6*3	CYP2D6.3	100CT; 1039CT; 1661GC; 1943GA; 4180GC;	CYP2D6*1 0D	P34S; R201H; S486T	.	.	Marez et al, 1997
15	CYP2D6*3	.	2587- 2590delGA CT	N2	Frameshift	None	.	Leathart et al, 1998
	CYP2D6*3	CYP2D6.3	1661GC; 4180GC	.	S486T	.	.	Submitted 17-Aug-00 by Dr. T. Shimada
20	CYP2D6*4	CYP2D6.4	1023CT; 1661GC; 1863 ins(TTT CGC CCC)2; 2850 CT; 4180GC	.	T107I; 172- 174(FRP)3 ; R296C; S486T	None (dx)	.	Submitted 28-Feb-01 by Dr. A. Gaedigk
	CYP2D6*4	CYP2D6.2	-1235AG; -740CT; - 678GA; 1661GC; 2850CT; 4180GC	.	R296C; S486T	Decr (s)	.	Raimundo et al, 2000 This allele is being further characteris ed.

b, bufuralol; d, debrisoquine; dx, dextromethorphan; s, sparteine  
SwissProtGenBankOMIMGeneCards

25 Note: The -1584CG; -1235AG; -740CT and -678GA polymorphisms are probably found in most alleles of the CYP2D6\*2 series (Raimundo et al, 2000).

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Table 18

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information			
5	CYP4A11 "cytochrome P450, subfamily IVA, polypeptide 11" (CP4Y, CYP4A2, CYP4A11)		NT_029224 (working draft chromo1)  NM_000778 (m) NP_000769 (p)	2815 bp 519 aa				
10								
Allelic Variants from SNP Database:								
	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
15	NT_029224	405284	rs2056899	XP_037166	a t	N Y	1	48
	NT_029224	405350	rs2056900	XP_037166	g a	G S	1	26

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Table 19

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP4F2</b> "cytochrome P450, subfamily IVF, polypeptide 2" (CPF2)	19pter- p13.11	NT_011281 (working draft chromo19) NT_025130 (working draft chromo19)  U02388 (m) NM_001082 (m) NP_001073 (p)	2360 bp 520 aa	

10

## Allelic Variants from SNP Database:

Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
NT_011281	77228	rs2108622	XP_051256	g a	V M	1	433

15

Table 20

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
20	<b>CYP11A</b> "cytochrome P450, subfamily XIA (cholesterol side chain cleavage)" (P450SCC, cytochrome P4 50C11A1)	15q23- q24	D00169  NT_010298 (g) M14565 (m) NM_000781 (m) NP_000772 (p)	1821 bp 521 aa	

25

## Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
30	NT_010298	262118	rs1130841	XP_007646	g a	C Y	2	16
	NT_010298	281261	rs1049968	XP_007646	c g	I M	3	301
35	NT_010298	281298	rs6161	XP_007646	g a	E K	1	314
	NT_010298	281298	rs6161	XP_027406	g a	E K	1	4

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Table 21

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP11B1</b> "cytochrome P450, subfamily XIB (steroid 11- beta-	8q21	D10169, D90428, X55765 (exon1 and 5' flanking region) D16153 (exon 1 and 2 normal) M32863, J05140 (exon 1 and 2) M32878 (exon 3-8) D16154 (exon 3-9) M32879 (exon 9) NT_008127 (working draft chromo8)	2092 bp 503 aa	
10	hydroxylase), polypeptide 1" (FHI, CPN1, CYP11B, P450C11)		NT_008127 (g) X55764 (m) NM_000497 (m) NP_000488 (p)		

15

**Allelic Variants from Scientific Literature:**

	DNA Variant	Protein Variant	Phenotype	References
	?	Pro42Ser	Steroid 11-Beta- hydroxylase deficiency	OMIM 202010
	?	Thr319Me t	Steroid 11-Beta- hydroxylase deficiency	OMIM 202010
	?	Asn133Hi s	Steroid 11-Beta- hydroxylase deficiency	OMIM 202010
20	?	Arg374Gl n	Steroid 11-Beta- hydroxylase deficiency	OMIM 202010
	?	Thr318Me t	Steroid 11-Beta- hydroxylase deficiency	OMIM 202010
	CGC > CAC	Arg448His	Steroid 11-Beta- hydroxylase deficiency	OMIM 202010

**Allelic Variants from SNP Database:**

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
25	NT_008127	147509	rs5294	XP_030748	t c	Y H	1	439
	NT_008127	147747	rs4541	XP_030748	c t	A V	2	386

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	NT_008127	147756	rs5312	XP_030748	a	E	2	383
					t	V		
	NT_008127	148261	rs6407	XP_030748	g	A	1	348
					a	T		
5	NT_008127	148788	rs5292	XP_030748	c	L	1	293
					g	V		
	NT_008127	148823	rs5291	XP_030748	g	S	2	281
					a	N		
	NT_008127	149180	rs5288	XP_030748	t	F	3	257
10					g	L		
	NT_008127	149208	rs4547	XP_030748	c	T	2	248
					t	I		
	NT_008127	149286	rs5308	XP_030748	a	N	2	222
					c	T		
15	NT_008127	149608	rs5287	XP_030748	g	M	3	160
				c	I			
	NT_008127	152097	rs5282	XP_030748	g	D	1	63
					c	H		
	NT_008127	152156	rs4534	XP_030748	g	R	2	43
20					a	Q		
	NT_008127	152255	rs6405	XP_030748	g	C	2	10
					a	Y		

Table 22

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
25	<b>CYP11B2</b>	8q21-q22	D13752	2936 bp 503 aa	
	"cytochrome P450, subfamily XIB (steroid 11- beta- hydroxylase), polypeptide 2" (CPN2, CYP11B, CYP11BL, P- 450C18, P450aldo)		X54741 (m) NM_000498 (m) NP_000489 (p)		
30					
35					

## Allelic Variants from Scientific Literature:

40	DNA Variant	Protein Variant	Phenotype	References
	?	Lys173Arg	Low renin, susceptibility to hypertension	OMIM 124080
	?	Glu198Asp	Congenital hypoaldosteronism	OMIM 124080
	?	Thr185Ile	Congenital hypoaldosteronism	OMIM 124080
	?	Leu461Pro	Congenital hypoaldosteronism	OMIM 124080

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GTG > GCG	Val386Ala	Congenital hypoaldosteronism	OMIM 124080
CGG > TGG	Arg181Tr p	Congenital hypoaldosteronism	OMIM 124080

Table 23

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP17</b> "cytochrome P450, subfamily XVII (steroid 17- alpha- hydroxylase), adrenal hyperplasia" (CPT7, S17AH, P450C17)	10q24.3	M19489  NT_029393 (g) M14564 (m) NM_000102 (m) NP_000093 (p)	1755 bp 508 aa	
10					
15					

## Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
	T > G	PHE417C YS	Alpha- hydroxylase/17,20- lyase deficiency	OMIM 202110
20	G > A	ARG358G LN	Alpha- hydroxylase/17,20- lyase deficiency	OMIM 202110
	G > A	ARG347H IS	Alpha- hydroxylase/17,20- lyase deficiency	OMIM 202110
	CGG > TGG	ARG96TR P	Alpha- hydroxylase/17,20- lyase deficiency	OMIM 202110
	CCA > ACA	PRO342T HR	Alpha- hydroxylase/17,20- lyase deficiency	OMIM 202110
	CGA > TGA	ARG239T ER	Alpha- hydroxylase/17,20- lyase deficiency	OMIM 202110
		SER106P RO	Adrenal hyperplasia	OMIM 202110

25

## Allelic Variants from SNP Database:

Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
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NT_029393	754865	rs762563	XP_005915	c g	C W	3	22
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Table 24

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP19</b>	15q21.1	L21982 (gene, untranslated exon 1.4)	3007 and 3116 bp 503 aa	
10	"cytochrome P450, subfamily XIX (aromatization of androgens)" (ARO, ARO1, CPV1, CYAR, P-450AROM)		NT_010204 (working draft chromo15)  NM_000103 (m) NM_031226 (m) NP_000094 (p) NP_112503 (p)		

## 15 Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
	C1303T	Arg435Cys	Aromatase deficiency	OMIM 107910
	G1310A	Cys437Tyr	Aromatase deficiency	OMIM 107910
	C1123T	Arg375Cys	Aromatase deficiency	OMIM 107910
20	G1094A	Arg365Gln	Aromatase deficiency	OMIM 107910

## Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
25	NT_010204	1691214	rs2236722	XP_035593	t	W	1	39
	NT_010204	1706104	rs1803154	XP_035593	a	R	1	108
	NT_010204	1718241	rs700519	XP_035593	t	*		
					c	R	1	264
					t	C		

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Table 25

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>CYP21A2</b> "cytochrome P450, subfamily XXIA (steroid 21- hydroxylase, congenital adrenal hyperplasia), polypeptide 2" (CPS1, CA21H, CYP21, CYP21B, P450C21B, P450c21B)	6p21.3	M13936  NG_000013 (g) NT_007592 (g) NM_000500 (m) M26856 (m) NP_000491 (p)	2112 bp 495 aa	

20

**Allelic Variants from Scientific Literature:**

DNA Variant	Protein Variant	Phenotype	References
	Gly424Ser	Adrenal Hyperplasia	OMIM 201910
	Glu380Asp	Adrenal Hyperplasia	OMIM 201910
	Arg339His	Adrenal Hyperplasia	OMIM 201910
	Met238Lys	Adrenal Hyperplasia	OMIM 201910
	Val236Glu	Adrenal Hyperplasia	OMIM 201910
	Ile235Asn	Adrenal Hyperplasia	OMIM 201910
	Tyr102Arg	21-Hydroxylase polymorphism	OMIM 201910
	Pro453Ser	Adrenal Hyperplasia	OMIM 201910
	Gly292Ser	Adrenal Hyperplasia	OMIM 201910
	Ser268Thr	21-Hydroxylase polymorphism	OMIM 201910
	Pro30Leu	Adrenal Hyperplasia	OMIM 201910
	Arg356Trp	Adrenal Hyperplasia	OMIM 201910
	Val281Leu	Adrenal Hyperplasia	OMIM 201910
	Ile172Asn	Adrenal Hyperplasia	OMIM 201910

**Allelic Variants from SNP Database:**

Contig Accession	Contig Position	dbSNPs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
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	NT_007592	8200835	rs6473	XP_004200	g	S	2	494
					a	N		
	NT_007592	8200956	rs6445	XP_004200	c	P	1	454
					t	S		
	NT_007592	8201852	rs6471	XP_004200	g	V	1	282
					t	L		
	NT_007592	8201890	rs6472	XP_004200	g	S	2	269
					c	T		
5	NT_007592	8202146	rs6476	XP_004200	t	M	2	240
					a	K		
	NT_007592	8202414	rs1040310	XP_004200	c	D	3	184
					g	E		
	NT_007592	8202536	rs6475	XP_004200	t	I	2	173
					a	N		
	NT_007592	8202853	rs6474	XP_004200	g	R	2	103
					a	K		
	NT_007592	8200835	rs6473	XP_042400	g	S	2	225
					a	N		
10	NT_007592	8200956	rs6445	XP_042400	c	P	1	185
					t	S		
	NT_007592	8201852	rs6471	XP_042400	g	V	1	13
					t	L		

Table 26

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
15	<b>CYP27A1</b> "cytochrome P450, subfamily XXVIIA (steroid 27- hydroxylase, cerebrotendino us xanthomatosis) , polypeptide 1" (CTX, CP27, CYP27)	2q33-qter	S62709 (5' region) NT_005289 (working draft chromo2)	2059 bp 531 aa	Cali <i>et al.</i> (1991) OMIM (213700) One mutation, called by them CTX1, was at codon 446 near the heme ligand, cys444. The second, called CTX2, was at codon 362 in the adrenodoxin binding region.
20			NM_000784 (m) NP_000775 (p)		
25					

## Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
30	G > A	Arg372Gln	Cerebrotendinous xanthomatosis	OMIM 213700
	C > T	Arg441Trp	Cerebrotendinous xanthomatosis	OMIM 213700

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5	G-to-A	Arg441Gln	Cerebrotendinous xanthomatosis	OMIM 213700
	(CGPy) to cysteine codons (TGPY).	Arg362Cys	Cerebrotendinous xanthomatosis	OMIM 213700
	(CGPy) to cysteine codons (TGPY).	Arg446Cys	Cerebrotendinous xanthomatosis	OMIM 213700

10

Table 27

15	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
	<b>CYP51</b> "cytochrome P450, 51 (lanosterol 14- alpha- demethylase)" (LDM, CP51, CYPL1, P450L1, P450- 14DM)	7q21.2- q21.3	AH006655  NT_029333 (g) NM_000786 (m) NP_000777 (p)	3381 bp	

20

## Allelic Variants from SNP Database:

25	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_029333	2609660	rs2229188	XP_004663	t c	V A	2	19

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Table 28

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information			
5	EPHX1 "epoxide hydrolase 1, microsomal (xenobiotic)" (MEH, EPHX)	1q42.1	AF253417, L29766, L25880  NT_004525 (g) NM_000120 (m) NP_000111 (p)	1856 bp 455 aa				
10	Allelic Variants from Scientific Literature:							
	DNA Variant	Protein Variant	Phenotype	References				
	?	Tyr113His	Epoxide hydrolase polymorphism, susceptibility to aflatoxin B1?	OMIM 132810				
	Allelic Variants from SNP Database:							
15	Contig Accession	Contig Position	dbSNPs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_004525	1595032	rs2234701	XP_001799	g a	R Q	2	454
	NT_004525	1595753	rs2137841	XP_001799	t a	H Q	3	387
	NT_004525	1601658	rs2234922	XP_001799	g a	R H	2	139
	NT_004525	1608433	rs1051740	XP_001799	c t	H Y	1	113
20	NT_004525	1611484	rs2234697	XP_001799	c t	R C	1	49

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Table 29

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	<b>EPHX2</b> (epoxide hydrolase 2, cytoplas)	8p21-p12	X97024 (exon 1) X97038 (exon 17, 18 and 19) NT_007988 (working draft chromo 8)  NM_001979 (m) L05779 (m) NP_001970 (p)	2100 bp 554 aa	

## Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
10	NT_007988	233832	rs751141	XP_005114	g a	R Q	2	287

Table 30

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
15	<b>GUSB</b> (glucuronidase, beta)	7q21.11	M65002 (5' end) Pseudogene AL021368 (BAC 55C20 on chromo6)  M15182 (m) NM_000181 (m) NP_000172 (p)	2191 bp 651 aa	

## Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
20	?	Trp446Ter	Mucopolysaccharido sis	
	?	Trp507Ter	Mucopolysaccharido sis	
	?	Tyr495Cy s	Mucopolysaccharido sis	
	?	Pro148Se r	Mucopolysaccharido sis	
	C1831T	Arg611Tr p	Mucopolysaccharido sis	
25	C1061T	Arg354Val	Mucopolysaccharido sis	

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C672T	Arg216Tr p	Mucopolysaccharido sis
C > T	Arg382Cy s	Mucopolysaccharido sis
C > T	Ala619Val	Mucopolysaccharido sis

Table 31

5	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
	<b>KCNH2</b>	7q35-q36	NT_007704 (working draft chromo7)	4070 bp 1159 aa	
10	"potassium voltage-gated channel, subfamily H (eag-related), member 2" (HERG, LQT2)		U04270 (m) NP_000229 (p)		

## 15 Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
	G1468A	Ala490Thr	Long QT syndrome	OMIM 152427
	?	Gly572Ar g	Long QT syndrome	OMIM 152427
	?	Arg582Cy s	Long QT syndrome	OMIM 152427
20	G1882A	Gly628Se r	Long QT syndrome	OMIM 152427
	G2647A	Val822Me t	Long QT syndrome	OMIM 152427
	T1961G	Ile593Arg	Long QT syndrome	OMIM 152427
	A1408G	Asn470As p	Long QT syndrome	OMIM 152427
	C1682T	Ala561Val	Long QT syndrome	OMIM 152427

## 25 Allelic Variants from SNP Database:

Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
NT_007704	8393	rs731506	XP_004743	t g	V G	2	41

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Table 32

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information			
5	LTA4H "leukotriene A4 hydrolase"	12q22	U27293 (exon 19 and complete cds.)  NM_000895 (m) J03459 (m) NP_000886 (p)	2060 bp 611 aa				
Allelic Variants from SNP Database:								
10	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_009685	277202	rs1803916	XP_012237	c g	T S	2	600

Table 33

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information			
15	<b>PTGIS</b> "prostaglandin I2 (prostacyclin) synthase" (CYP8, PGIS, PTGI, CYP8A1)	20q13.11- q13.13	D83393 (exon1) NT_011362 (working draft chromo20)  NP_000952 (m) NP_000952 (p)	5605 bp 500 aa				
20								
Allelic Variants from SNP Database:								
25	Contig Accession	Contig Position	dbSNPrs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
	NT_011362	1317708 1	rs5584	XP_030507	c	P	1	500
	NT_011362	1319336 3	rs5626	XP_030507	t c	S R	1	236
	NT_011362	1321347 1	rs5624	XP_030507	t t	C F	1	171
	NT_011362	1321352 1	rs5623	XP_030507	c a	L E	2	154
	NT_011362	1321702 0	rs5622	XP_030507	c t	A S	3	118
					a	R		



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Table 34

	Gene (Synonyms)	Locus	Accession #s	mRNA / Protein	Structural Information
5	TPMT "thiopurine S- methyltransferase"	6p22.3	U81562  NT_007180 (g) NM_000367 (m) S62904 (m) NP_000358 (p)	2742 bp 245 aa	

## Allelic Variants from Scientific Literature:

	DNA Variant	Protein Variant	Phenotype	References
10	A719G	Tyr240Cys	6-mercaptopurine sensitivity	OMIM 187680
	G644A	Arg215His	6-mercaptopurine sensitivity	OMIM 187680
	G460A	Ala154Thr	6-mercaptopurine sensitivity	OMIM 187680
	G238C	Ala80Pro	6-mercaptopurine sensitivity	OMIM 187680

## Allelic Variants from SNP Database:

	Contig Accession	Contig Position	dbSNPs# (Cluster ID)	Protein Accession	dbSNP Allele	Protein Residue	Codon Position	Amino Acid
15	NT_007180	151037	rs1800462	XP_012752	g	A	1	80
					c	P		
	NT_007180	164074	rs1142345	XP_012752	a	Y	2	240
20					g	C		

## Table references

	Cascorbi <i>et al.</i> , <i>Clin. Pharmacol. Ther.</i> 69:169-174 (2001)
	Choi <i>et al.</i> , <i>Cell</i> 53:519-529 (1988)
	Hoffmeyer <i>et al.</i> , <i>Proc. Natl. Acad. Sci. USA</i> 97:3473-3478 (2000)
25	Ito <i>et al.</i> , <i>Pharmacogenetics</i> 11:175-184 (2001)
	Mickley <i>et al.</i> , <i>Blood</i> 91:1749-1756 (1998)
	Safa <i>et al.</i> , <i>Proc. Natl. Acad. Sci. USA</i> 87:7225-7229 (1990)
	Tanabe <i>et al.</i> , <i>J. Pharmacol. Exp. Ther.</i> 297:1137-1143 (2001)
	Toh <i>et al.</i> , <i>Amer. J. Hum. Genet.</i> 64:739-746 (1999)
30	Wada <i>et al.</i> , <i>Hum. Mol. Genet.</i> 7:203-207(1998)

**Table 35**  
BCR-ABL Kinase Domain Mutations Affecting Response to Imatinib

Mutation	Phase of disease*	Proposed mechanism of resistance
M244V	CP	impairs conformational change (P loop)
G250E	MBC	impairs conformational change (P loop)
Q252H/R	MBC	impairs conformational change (P loop)
Y253F/H	MBC, LBC	impairs conformational change (P loop)
E255K	MBC, LBC, CP, P-MBC	impairs conformational change (P loop)
T315I	MBC, LBC, CP, P-MBC	directly affects imatinib binding
F317L	MBC, CP	directly affects imatinib binding
M351T	MBC, LBC, CP	impairs conformational change (adjacent to activation loop)
E355G	MBC	impairs conformational change (adjacent to activation loop)
F359V	MBC, CP	directly affects imatinib binding
V379I	CP-NCR	impairs conformational change (activation loop)
L387M	CP	impairs conformational change (activation loop)
H396R	MBC, CP	impairs conformational change (activation loop)

\* MBC: myeloid blast crisis

LBC: lymphoid blast crisis

CP: chronic phase

CP-NCR: chronic phase with hematologic response in the absence of cytogenetic response (cytogenetic nonresponder)

**Table 36**

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## Beta tubulin (Isoform M40) Mutations Affecting Response to Paclitaxel

DNA Variant	Protein Variant	Phenotype	Reference
T810G	Phe270Val	paclitaxel resistance	Gianakakou <i>et al.</i> , <i>J. Biol. Chem.</i> 272:17118-25 (1997)
G1092A	Ala364Thr	paclitaxel resistance	Gianakakou <i>et al.</i> , <i>J. Biol. Chem.</i> 272:17118-25 (1997)

5                   The collections of the present invention require that the genotypically distinct coisogenic cells be in sufficient spatial proximity to one another as readily and contemporaneously to be subject to a common experimental protocol, yet remain separately assayable.

10                   Separate assayability can easily be effected by maintaining each of the genotypically distinct coisogenic cells of the collection in fluid noncommunication with the others of the cells of the collection. Spatial proximity can be effected by disposing the cells within wells or other types of fluidly noncommunicating locations that are within or upon a common structure.

15                   For example, each genotypically distinct cell (typically, cell line) can be disposed in a well (or wells) of a microtiter plate distinct from the well (or wells) in which genotypically-distinct cells are placed. Microtiter plates are now readily available commercially that have 24, 96, 384, 864, 1536, 3456, 6144, and 9600 wells. And variants abound. For example, U.S. Patent No. 6,171,780 B1 describes low fluorescence multiwell platforms for cellular screening assays. U.S. Patent No. 6,103,479 describes methods  
20                   apparatus for non-uniform micro-patterned arrays of cells. Chiu *et al.*, *Proc. Natl. Acad. Sci. USA* 97(6):2408-13 (2000) describe the patterned deposition of cells onto surfaces by using three-dimensional microfluidic systems. A wide variety of "chip-based", microfluidic devices for arraying cells are also now described. See, e.g., U.S. Patent No. 6,086,740 ("Multiplexed microfluidic devices and systems").

25                   Alternatively, the genotypically distinct cells of the collection can be maintained in fluid noncommunication by disposing each genotypically distinct cell (typically, as a genotypically distinct cell line) in a separate structurally discrete, fluidly noncommunicating container, such as a vial, ampule, or tube; spatial proximity can in such

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cases be effected by packaging the separate containers together. In such cases, the cell collections of the present invention take the form of a kit, and it is therefore another aspect of the present invention to provide kits comprising the coisogenic cell collections of the present invention.

5           The kits comprise at least five genotypically distinct cells, the cells contained within separate, structurally discrete, fluidly noncommunicating containers; the at least five structurally discrete containers are packaged together. As described above, each of the at least 5 genotypically distinct cells is coisogenic with respect the others of the at least 5 genotypically distinct cells at a target locus common thereamong.

10           Since the cell collections of the present invention can include a great many more than five genotypically distinct cells, the kits of the present invention can usefully and additionally include computer-readable media having at least one dataset that defines the genotype of the cells of the collection at least at the target locus; the dataset can usefully include links to extrinsic databases, such as the Online Mendelian Inheritance of Man  
15 (OMIM) (<http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=OMIM>), the Human Gene Mutation Database (HGMD) (<http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>), or more general databases, such as GenBank, or the UCSC human genome project working draft (<http://genome.ucsc.edu/>).

20           Fluid noncommunication is not required where the genotypically distinct cells can be distinguished even in admixture. In such case, the cells can be contained in a common container, such as a tube, ampule, well, or dish; the required spatial proximity is of course thus necessarily maintained.

25           For example, if the assay measures cell proliferation under a chosen condition, such as exposure to a chemotherapeutic agent, *e.g.* paclitaxel or a derivative thereof, and the cells are individually bar coded, the cells can be commonly cultured in the presence of the drug agent, and the degree of individual proliferation assessed by stoichiometric amplification and quantification of their respective bar codes. See, *e.g.*, U.S. Patent No. 6,046,002, incorporated herein by reference in its entirety.

30           Additionally, the coisogenic cell collections of the present invention need not be in a form that can immediately be assayed. Rather, the collections can be provided in any physical form that will, at some point, permit the genotypically distinct cultured cells

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separately to be assayed. In one embodiment, for example, the cells can be provided frozen, either in individual tubes or ampules or collectively in the wells of a microtiter dish, thereafter to be thawed, propagated, and assayed. Where the cells are yeast cells, the cells can conveniently be provided frozen or lyophilized.

5                   The invention further provides, in another aspect, methods of making the coisogenic cell collections of the present invention.

                  In a basic embodiment, the method comprises collecting at least 5 genotypically distinct cells, each of the cells being coisogenic with respect to the others of the at least 5 genotypically distinct cells at a target locus common thereamong, into a  
10   collection in which each of the genotypically distinct cells can be separately assayed.

                  Typically, but not invariably, the method further comprises the earlier step of making cells that are coisogenic at a common target locus. The coisogenic cells are made by engineering, into at least four of at least five cultured cells, the cells derived from a common eukaryotic ancestor cell, a genomic sequence alteration at a target locus common  
15   thereamong; the sequence alterations must be sufficient to cause at least five distinct protein sequences collectively to be encoded by the cells at the common target locus.

                  The genomic sequence alterations can be created by any means that permits mutations to be targeted to genomic sequence. In a presently preferred approach, mutations are targeted to a common target locus using modified single-stranded  
20   oligonucleotides ("targeting oligonucleotides").

                  We have recently described methods for targeting single nucleotide changes directly into long pieces of genomic DNA present within YACs, BACs, and even intact cellular chromosomes through use of sequence-altering oligonucleotides. See international patent publication nos. WO 01/73002, WO 01/92512, and WO 02/10364; and  
25   commonly owned and copending U.S. provisional patent application nos. 60/326,041, filed September 27, 2001, 60/337,129, filed December 4, 2001, 60/393,330, filed July 1, 2002, 60/363,341, filed March 7, 2002; 60/363,053, filed March 7, 2002, and 60/363,054, filed March 7, 2002, the disclosures of which are incorporated herein by reference in their entireties. These methods, described in further detail below, are presently preferred.

30                   Other approaches for targeting sequence changes using sequence altering oligonucleotides have also been described. See e.g. U.S. Patent Nos. 6,303,376;

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5,776,744; 6,200,812; 6,074,853; 5,948,653; 6,136,601; 6,010,907; 5,888,983; 5,871,984; 5,760,012; 5,756,325; and 5,565,350, the disclosures of which are incorporated herein by reference in their entireties. These latter approaches typically have lower efficiency and are at present less preferred, although they may at times be used.

5 Changes can be targeted directly into cellular chromosomes within cultured eukaryotic cells. In other embodiments, changes can instead be targeted to recombinant constructs *in vitro*, with the modified target thereafter used to integrate the desired change into a cultured eukaryotic cell.

The first of these approaches is particularly preferred for creating  
10 coisogenic cell collections that are legacy-free, and/or exceptionally or perfectly coisogenic. The second approach is preferred, *inter alia*, in construction of coisogenic cell collections having identical targeted changes superimposed on different genetic backgrounds.

In the latter approach, the vector is usefully an artificial chromosome, such as YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes), PACs  
15 (P-1 derived artificial chromosomes), HACs (human artificial chromosomes), and PLACs (plant artificial chromosomes).

Artificial chromosomes are reviewed in Larin *et al.*, *Trends Genet.* 18(6):313-9 (2002); Choi *et al.*, *Methods Mol. Biol.* 175:57-68 (2001); Brune *et al.*, *Trends Genet.* 16(6):254-9 (2001); Ascenzioni *et al.*, *Cancer Lett.* 118(2):135-42 (1997); Fabb *et al.*, *Mol. Cell. Biol. Hum. Dis. Ser.* 5:104-24 (1995); Huxley, *Gene Ther.* 1(1):7-12 (1994),  
20 the disclosures of which are incorporated herein by reference in their entireties. Other vectors that may be used include viral, typically eukaryotic viral, vectors, such as adenoviral, varicella, and herpesvirus vectors.

Yeast artificial chromosomes (YACs) are additionally described in Burke *et al.*, *Science* 236:806; Peterson *et al.*, *Trends Genet.* 13:61 (1997); Choi *et al.*, *Nature Genet.*, 4:117-223 (1993); Davies *et al.*, *Biotechnology* 11:911-914 (1993); Matsuura *et al.*, *Hum. Mol. Genet.*, 5:451-459 (1996); Peterson *et al.*, *Proc. Natl. Acad. Sci.*, 93:6605-6609 (1996); and Schedl *et al.*, *Cell*, 86:71-82 (1996)). Human artificial chromosomes (HACs) are additionally described in Kuroiwa *et al.*, *Nature Biotechnol.* 18(10):1086-90 (2000);  
25 Henning *et al.*, *Proc. Natl. Acad. Sci. USA* 96(2):592-7 (1999); Harrington *et al.*, *Nature Genet.* 15(4):345-55 (1997). Bacterial artificial chromosomes (BACs) and P-1 derived  
30

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artificial chromosomes (PACs) are further described in Mejia *et al.*, *Genome Res.* 7:179-186 (1997); Shizuya *et al.*, *Proc. Natl. Acad. Sci.* 89:8794-8797 (1992); Ioannou *et al.*, *Nature Genet.*, 6:84-89 (1994); Hosoda *et al.*, *Nucleic Acids Res.* 18:3863 (1990). Other vectors useful in the present invention are further described in Sternberg *et al.*, *Proc. Natl. Acad. Sci. USA* 87:103-107 (1990).

BACs have been developed for transformation of plants with high-molecular weight DNA using the T-DNA system (Hamilton, *Gene* 24:107-116 (1997); Frary *et al.*, *Transgenic Res.* 10: 121-132 (2001)).

In certain useful embodiments, genomic targets are present within vectors that permit integration of the target into a cellular chromosome. In particularly useful embodiments, genomic targets are present within vectors that permit site-directed integration of the target into a cellular chromosome. Usefully, the vector is an artificial chromosome and site-specific integration may be performed by recombinase mediated cassette exchange (RMCE).

In RMCE, a region of DNA (cassette) desired to be integrated into a specific cellular chromosomal location is flanked in a recombinant vector by sites that are recognized by a site-specific recombinase, such as loxP sites and derivatives thereof for Cre recombinase and FRT sites and derivatives thereof for Flp recombinase. Other site-specific recombinases having cognate recognition/recombination sites useful in such methods are known (see, e.g., Blake *et al.*, *Mol. Microbiol.* 23(2):387-98 (1997)).

The site in the cellular chromosome into which the cassette is desired site-specifically to be integrated is analogously flanked by recognition sites for the same recombinase.

To favor a double-reciprocal crossover exchange reaction between vector and chromosome, two approaches are typical. In the first, the two sites (such as lox or FRT) that flank the cassettes in both vector and cellular chromosome are heterospecific: that is, they differ from one another and recombine with each other with far lower efficiency than with sites identical to themselves. In the second, the lox or FRT sites are inverted. See, e.g., Baer *et al.*, *Curr. Opin. Biotechnol.* 12:473-480 (2001); Langer *et al.*, *Nucl. Acids Res.* 30:3067-3077 (2002); Feng *et al.*, *J. Mol. Biol.* 292:779-785 (1999), the disclosures of which are incorporated herein by reference in their entireties.

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Recombinational exchange of the cassettes from vector to cellular chromosome, with integration of the construct cassette site-specifically into the cellular chromosome, is effected by introducing the recombinant construct into the cell and expressing the site-specific recombinase appropriate to the recombination sites used. The site-specific recombinase may be expressed transiently or continuously, either from an episome or from a construct integrated into cellular chromosome, using techniques well known in the art.

Site-specific recombinational insertion provides a single-copy integrant of defined and chosen sequence in a defined cellular genomic milieu. It is known that such site-specific integration provides more consistent expression than does random integration. Feng *et al.*, *J. Mol. Biol.* 292:779-285 (1999).

Our presently preferred methods for targeting single nucleotide changes directly into genomic DNA -- whether targeted directly into a eukaryotic chromosome or first targeted into a recombinant construct in vitro -- are further described in international patent publication nos. WO 01/73002, WO 01/92512, and WO 02/10364; and commonly owned and copending U.S. provisional patent application nos. 60/326,041, filed September 27, 2001, 60/337,129, filed December 4, 2001, 60/393,330, filed July 1, 2002, 60/363,341, filed March 7, 2002; 60/363,053, filed March 7, 2002, and 60/363,054, filed March 7, 2002; the disclosures of which are incorporated herein by reference in their entireties.

Briefly, the method comprises combining the targeted nucleic acid, in the presence of cellular repair proteins, with a single-stranded oligonucleotide 17 - 121 nucleotides in length, the oligonucleotide having an internally unduplexed domain of at least 8 contiguous deoxyribonucleotides. The oligonucleotide is fully complementary in sequence to the sequence of a first strand of the nucleic acid target, but for one or more mismatches as between the sequences of the internally unduplexed deoxyribonucleotide domain and its complement on the target nucleic acid first strand. Each of the mismatches is positioned at least 8 nucleotides from each of the oligonucleotide's 5' and 3' termini, and the oligonucleotide has at least one terminal modification.

The oligonucleotide terminal modification is typically selected from the group consisting of at least one terminal locked nucleic acid (LNA), at least one terminal 2'-O-Me base analog, and at least three terminal phosphorothioate linkages.



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LNAs are bicyclic and tricyclic nucleoside and nucleotide analogs and the oligonucleotides that contain such analogs. The basic structural and functional characteristics of LNAs and related analogues that usefully may be incorporated into the second ("annealing") oligonucleotide in the methods of the present invention are disclosed

5 in various publications and patents, including WO 99/14226, WO 00/56748, WO 00/66604, WO 98/39352, U.S. Patent No. 6,043,060, and U.S. Patent No. 6,268,490, the disclosures of which are incorporated herein by reference in their entireties. See also Singh *et al.*, *Chem. Commun.* 1998: 455; Koshkin *et al.*, *Tetrahedron* 54:3607 (1998); Koshkin *et al.*, *Tetrahedron Lett.* 39:4381 (1998); Singh *et al.*, *Chem. Commun.* 1998:1247, and are

10 reviewed in Orum *et al.*, "Locked nucleic acids: a promising molecular family for gene-function analysis and antisense drug development," *Curr. Opin. Mol. Ther.* 3(3):239-43 (2001), the disclosures of which are incorporated herein by reference in their entireties.

Synthesis of LNA nucleosides and nucleoside analogs and oligonucleotides

15 that contain them may be performed as disclosed in WO 99/14226, WO 00/56748, WO 00/66604, WO 98/39352, U.S. Patent No. 6,043,060, and U.S. Patent No. 6,268,490. Many may now be ordered commercially (Exiqon, Inc., Vedbaek, Denmark; Proligo LLC, Boulder, CO, USA).

The oligonucleotides are typically at least 17 nucleotides in length, and can

20 usefully be up to about 121 nucleotides in length, and even longer, although targeting oligonucleotides of about 17 to about 74 nucleotides in length are at present preferred. The oligonucleotides used to create the coisogenic cell collections may thus have lengths of 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65,

25 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, or 121 nt.

At present most preferred are targeting oligonucleotides at least about 25 bases in length, unless there are self-dimerization structures within the oligonucleotide; if

30 the oligonucleotide has such an unfavorable structure, lengths longer than 35 bases are preferred.

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The internally unduplexed alteration domain of the targeting oligonucleotide is preferably fully complementary to one strand of the target locus, except for the mismatched base (or up to about 3 mismatched bases) introduced to effect the gene alteration or conversion events. The central alteration domain is generally at least

5 8 nucleotides in length. Although it is presently preferred to locate the alteration domain approximately in the middle of the targeting oligonucleotide, there is no strict requirement for symmetrical extension adjacent to the alteration DNA domain. However, the base(s) targeted for alteration in the most preferred embodiments are at least about 8, 9 or 10 bases from each of the ends of the targeting oligonucleotide.

10 The targeting oligonucleotide preferably binds to the non-transcribed strand of a genomic DNA duplex.

The oligonucleotides used to make the coisogenic cell collections of the present invention preferably contain more than one of the aforementioned modifications ("backbone modifications"), preferably (but not obligately) at both ends of the

15 oligonucleotide. In some embodiments, the backbone modifications are adjacent to one another. For oligonucleotides of the invention that are longer than about 17 to about 25 bases in length, internal as well as terminal region segments of the backbone can be altered.

The optimal number and placement of backbone modifications for any

20 individual oligonucleotide will vary with the length of the oligonucleotide and the particular type of backbone modification(s) that are used, and may be determined by routine comparative studies, as further described in WO 01/73002 and commonly owned and copending U.S. patent application serial no. 09/818,875, filed March 27, 2001, the disclosures of which are incorporated herein by reference in their entireties.

25 The sequence-altering oligonucleotide can be contacted to its genomic target within intact cells, within cell-free protein extracts having cellular repair proteins, or within purified protein fractions having cellular repair proteins.

Efficiency of conversion is defined herein as the percentage of recovered substrate molecules that have undergone a conversion event. Depending on the nature of

30 the target genetic material, e.g. the genome of a cell or a genomic construct in a replicable vector, efficiency can be represented as the proportion of cells or clones containing an

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extrachromosomal element that exhibit a particular phenotype. Alternatively, representative samples of the target genetic material can be sequenced to determine the percentage that have acquired the desired change.

Efficiency can be increased using the methods set forth in commonly  
5 owned and copending U.S. provisional application serial nos. 60/363,341, filed March 7, 2002; 60/363,053, filed March 7, 2002; and 60/363,054, filed March 7, 2002, the disclosures of which are incorporated herein by reference in their entireties.

In the first of these methods, the eukaryotic cell to be targeted, or that provides the protein extract having cellular repair enzymes within which a recombinant  
10 construct is targeted, is first contacted with an inhibitor of histone deacetylase (HDAC), such as Trichostatin A. In the second of these methods, the sequence-altering oligonucleotide is contacted with the genomic target – either within a cell or within a cell extract – in the presence of lambda beta protein. In the third of these methods, the eukaryotic cell to be targeted, or that provides the protein extract within which a recombinant construct is  
15 targeted, is first contacted with hydroxyurea.

Targeting efficiency may also be increased using the methods set forth in U.S. provisional patent application serial nos. 60/325,992, filed September 27, 2001; 60/337,129, filed December 4, 2001; and 60/393,330, filed July 1, 2002, the disclosures of which are incorporated herein by reference in their entireties, and in U.S. provisional  
20 application serial nos. 60/220,999, filed July 27, 2000; and 60/244,989, filed October 30, 2000, the disclosures of which are incorporated herein by reference in their entireties.

In various of these methods, the cell or cell-free extract within which targeting is performed has altered levels or activity of at least one protein from the RAD52 epistasis group, the mismatch repair group or the nucleotide excision repair group, such as  
25 reduced levels or activity of at least one protein selected from the group consisting of a homolog, ortholog or paralog of RAD1, RAD51, RAD52, RAD57 and PMS1.

In others of these methods, the cell or cell-free extract within which targeting is performed has increased levels or activity of at least one of RAD10, RAD51, RAD52, RAD54, RAD55, MRE11, PMS1 or XRS2 proteins and decreased levels or activity  
30 of at least one other protein selected from the group consisting of RAD1, RAD51, RAD52, RAD57 or PMS1.

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The targeting oligonucleotides can introduce more than a single base change in a single step. For example, in an oligonucleotide that is about a 70-mer, with at least one modified residue incorporated on each of the two ends, multiple bases up to 27 nucleotides apart can be targeted. However, when the targeting oligonucleotide includes  
5 multiple sequence changes, not all transformants will include all genetic changes: there is a frequency distribution such that the closer the target bases are to each other in the alteration domain, the higher the frequency of change in a given cell. Target bases only two nucleotides apart are changed together in every case that has been analyzed. The farther apart the two target bases are, the less frequent the simultaneous change.

10 Thus, in creating the coisogenic cell collections of the present invention, targeting oligonucleotides can be used to alter multiple bases at the target locus, rather than just a single base. Furthermore, iterative rounds of targeting can be performed to introduce multiple changes.

15 In embodiments in which the genome is targeted directly in the cell, the targeting oligonucleotides can be introduced into the cell by any means known in the art, such as through use of polycations, cationic lipids, liposomes, polyethylenimine (PEI), electroporation, biolistics, microinjection and other methods known in the art to facilitate cellular uptake; indeed, at times the targeting oligonucleotides can be introduced by simple incubation without any adjunctive means.

20 In alternative embodiments, the targeting oligonucleotide can be used to introduce the alteration into a genomic DNA construct, with the altered construct thereafter introduced into the cells by known transfection techniques. Typically, the altered construct is far larger than the targeting oligonucleotide, and is sufficient in length to act as a substrate for subsequent homologous recombination with the cellular chromosome.

25 The coisogenic cell collections of the present invention are useful for screening for the phenotypic effects of changes in the protein sequence encoded at a target locus. Because the cells of the collection are coisogenic, phenotypic differences detected among the cells of the collection can more reliably be ascribed to the differences in sequence at the target locus than in assays using genetically more heterogeneous cells in  
30 which additional changes at the target locus, or further changes at loci other than the target locus, can confound the analysis. Furthermore, given the ability readily to include within the

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collection of the present invention coisogenic cells that collectively have changes at many (including all) of the amino acids encoded at the target locus, the coisogenic cell collections of the present invention are extremely useful for dissecting structure activity relationships within proteins.

5                   Thus, in another aspect, the invention provides a method of identifying genotypes of a target locus that alter a cellular phenotype.

                  The method comprises assaying each genotypically distinct cell of a coisogenic cell collection of the present invention for a common phenotypic characteristic; the genotypically distinct cells are coisogenic at a desired target locus. From the assay  
10 results, at least one genotypically distinct cell is identified within the collection that has an alteration in the assayed phenotypic characteristic (*i.e.*, that exhibits an altered phenotype). Assay results are correlated with the target locus genotype, the correlation identifying genotypes of the target locus that cause an alteration of the cellular phenotype.

                  The phenotypic characteristic can be any cellular characteristic relevant to  
15 the target locus that can be assayed *in vitro*. A wide variety of such *in vitro* assays exist, and the principles for design of such assays are by now well known; accordingly, details will not here be presented.

                  Briefly, however, and solely by way of example, where the target locus is, for example, a steroid receptor, the phenotypic characteristic can be the detectable  
20 translocation of the receptor from cytoplasm to nucleus upon contact of the cells to the receptor's cognate ligand, as is described, *inter alia*, in U.S. Patent No. 5,989,835. The phenotypic characteristic where the target locus encodes a steroid hormone receptor can alternatively (or additionally) be the expression of a detectable reporter, such as a  
25 fluorescent protein (*e.g.*, GFP), driven from a hormone-responsive promoter. In this latter case, the assay depends upon the presence commonly within the cells of the coisogenic collection of a recombinant reporter construct. The recombinant construct can be present within the cells either on an episome or, usefully, integrated into the cellular genome at a locus elsewhere than at the target locus.

                  Where the target locus encodes a protein known to affect drug  
30 responsiveness, such as those described in detail above, the cellular characteristic to be assayed can be as simple and fundamental as degree of cell death, or can alternatively (or

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additionally) be, for example, the degree of cellular proliferation, degree of metabolic activity, and/or the degree of apoptosis. Appropriate assays are described in several compendia, such as Apoptosis and Cell Proliferation, 2<sup>nd</sup> ed., Boehringer Mannheim, 1998 (available on-line at

- 5 [http://biochem.boehringer-mannheim.com/prod\\_inf/manuals/cell\\_man/acp.pdf](http://biochem.boehringer-mannheim.com/prod_inf/manuals/cell_man/acp.pdf)), and Poirier (ed.), Apoptosis Techniques and Protocols, Humana Press, 1997 (ISBN: 0896034518), the disclosures of which are incorporated herein by reference. In addition, a wide variety of assay kits are available commercially (e.g., the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay, catalogue no. G5421, Promega, Madison, WI, which is a
- 10 colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays; the Apoptosis Detection System, Fluorescein, catalogue no. G3250, and the DeadEnd™ Colorimetric Apoptosis Detection System, catalogue no. G7360, both from Promega, Madison, WI; ApoAlert™ Apoptosis Detection Kits, Clontech Labs, Palo Alto, CA, USA).

- 15 Where the target locus encodes a protein known to affect drug responsiveness by transport of the drug from the cell interior to the medium, the characteristic to be assayed can alternatively, or additionally, be accumulation or efflux of the drug of interest or proxy therefor. Assays are now well known that permit such accumulation and/or efflux to be measured.

- 20 For example, U.S. Patent Nos. 6,277,655 and 5,872,014, incorporated herein by reference in their entireties, describe assays for activity of ABCB1 (MDR1) based upon fluorescent detection of the degree of cellular accumulation of free calcein after exposure to an acetoxymethyl ester or acetate ester of calcein. Ludescher *et al.*, *Br. J. Haematol.* 82(1):161-8 (1992) describe a flow cytometric assay for ABCB1 activity based
- 25 upon degree of intracellular accumulation of rhodamine 123. Gheuens *et al.*, *Cytometry* 12(7):636-44 (1991), describe flow cytometric double labeling techniques for assay of multidrug resistance. Cano-Gauci *et al.*, *Biochem. Biophys. Res. Commun.* 167(1):48-53 (1990) describe a fast kinetic analysis assay for drug transport in multidrug resistant cells using a pulsed quench-flow apparatus. Van Acker *et al.*, *Leukemia* 9:1398-406 (1995)
- 30 describe a rapid flow cytometric functional assay for P-glycoprotein (encoded by ABCB1) using fluo-3. Other assays are reviewed in Hoffman, "In vitro assays for chemotherapy

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sensitivity," *Crit. Rev. Oncol. Hematol.* 15(2):99-111 (1993); Cree *et al.*, "Tumor chemosensitivity and chemoresistance assays," *Cancer* 78(9):2031-2 (1996).

The assay can detect a phenotypic characteristic under static environmental conditions, or can instead can detect a phenotypic characteristic during or  
5 after an alteration in the cellular environment. In a useful embodiment of this latter approach, the coisogenic collection of cells is first exposed to a xenobiotic, usefully a known or potential therapeutic agent, and a characteristic of the cells measured thereafter.

Analogously, the assay can detect an equilibrium or otherwise static aspect of the phenotypic characteristic, or can detect kinetic changes in the phenotypic  
10 characteristic. For example, in an assay for cytoplasm to nuclear translocation of a steroid receptor, the assay can measure the static nuclear:cytoplasmic ratio of the receptor or can, in the alternative or in addition, measure the rate of translocation from cytoplasm to nucleus.

The assay can be quantitative or qualitative, manual or automated.

From the assay results, at least one cell is identified that has an altered  
15 cellular phenotype.

As would be well understood, not all genotypic changes at the target locus will affect the measured phenotypic characteristic. In order, however, to identify residues of the target protein whose change (by way of substitution, deletion, elimination by truncation, etc.) affects a phenotypic characteristic, at least one cell must be identified that has an  
20 alteration in the assayed phenotypic characteristic.

That said, data on residues of the protein encoded at the target locus that are tolerant of substitution are also tremendously useful, and in another aspect, therefore, the invention provides the converse method, in which residues tolerant of alteration are identified; in this latter method, correlation of the target locus genotype of cells that do not  
25 exhibit change in the assayed phenotypic characteristic identifies residues tolerant of substitution.

As would be readily understood, the "altered phenotype" is altered relative to a chosen control. The control is typically a coisogenic cell, typically in the same collection, that has a desired reference target locus sequence. The desired reference target  
30 locus sequence can, for example, be that of the parent cell (typically, cell line) from which the coisogenic cells of the collection have been engineered; that which is most commonly

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observed in a given population (e.g., the predominant allelic variant of the target locus in a chosen human population); or one chosen based upon prior-determined results of a phenotypic assay.

Following the assay, the results of the phenotypic assay are correlated with  
5 the cells' respective target locus genotypes.

The correlation can be performed either before or after identifying, from the assay results, at least one cell with altered cellular phenotype. If performed after the subset with altered phenotypic characteristic is identified, the correlation of phenotype with target locus genotype can be limited to that subset; if performed before the subset with altered  
10 phenotype is identified, as would typically be the case in high throughput applications of the methods of the present invention, the correlation of phenotype with target locus genotype would typically be made for all cells of the coisogenic cell collection.

In either case, the correlation of the subset's phenotypic assay results with their respective target locus genotypes identifies those genotypes of the target locus that  
15 cause an alteration of the cellular phenotype.

Correlation can be as simple as noting a change in phenotype for a given genotype, such as an increase in cytotoxicity occasioned by contact with a chemotherapeutic agent in a cell having a change in a specific ABCB1 amino acid. Alternatively, or in addition, correlation can be performed using statistical algorithms known  
20 in the art.

Where the coisogenic cell collection includes cells that collectively include changes at each amino acid of the protein encoded at the target locus (typically excluding changes of the initiator methionine), correlation of phenotype with genotype can identify all residues of the protein that are critical to its function. Where the coisogenic cell collection  
25 includes cells that collectively include each of the 20 natural amino acids at a single residue location, typically a residue previously shown or suspected to contribute to protein function, correlation of phenotype with genotype can identify with precision the structural requirements for function at that residue. Where the coisogenic cell collection includes one or more cells that have a naturally-occurring allelic variant of the target locus, or that encode  
30 a protein having a sequence identical to that encoded by a naturally-occurring allelic variant



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of the target locus, correlation of phenotype with genotype allows the phenotypic effects of such natural variants readily to be assessed in the context of a uniform genetic background.

In one series of embodiments, the method is used to identify genotypes that alter the cellular responsiveness to xenobiotics, which will typically be known or  
5 potential therapeutic agents.

In such embodiments, as well as in other embodiments of the methods of the present invention, the target locus at which the cells of the collection are coisogenic can usefully be selected from the group consisting of: CYP1A2, CYP2C17, CYP2D6, CYP2E, CYP3A4, CYP4A11, CYP1B1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9,  
10 CYP11A, CYP2C19, CYP2F1, CYP2J2, CYP3A5, CYP3A7, CYP4B1, CYP4F2, CYP4F3, CYP6D1, CYP6F1, CYP7A1, CYP8, CYP11A, CYP11B1, CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, CYP51, ABCB1, ABCB4, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, MRP7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, EPHX1, EPHX2, LTA4H, TRAG3, GUSB, TMPT, BCRP, HERG, hKCNE2, UDP glucuronosyl transferase  
15 (UGT), sulfotransferase, sulfatase, glutathione S-transferase (GST) -alpha, glutathione S-transferase -mu, glutathione S-transferase -pi, ACE, and KCHN2.

The method can usefully include a step, before assay, of contacting the coisogenic cell collection with a xenobiotic, typically a known or potential therapeutic agent. Potential therapeutic agents can be natural products or products of a combinatorial  
20 chemical synthesis.

The method can also usefully include a later step, after the correlations have been made, of collecting the correlations into at least one dataset; the dataset is often, but not necessarily, recorded on a computer-readable medium. In such case, the dataset can thereafter usefully be queried, e.g. to predict a cellular phenotype based upon the  
25 genotype at the relevant target locus.

Thus, in another aspect, the invention provides a method of predicting a phenotypic characteristic of a cell based upon its genotype at a target locus. The method comprises using the cell's genotype at a chosen target locus, or a unique identifier thereof, as a query to retrieve from a dataset data that report a phenotypic characteristic correlated  
30 with the target locus genotype. The dataset that is queried in this method includes correlations from at least five cells that are coisogenic at the target locus. The phenotypic

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characteristic retrieved from query of the dataset provides a prediction of the cell's phenotypic characteristic.

The target locus "genotype" to be used as a query can be obtained by any means known in the art, including sequencing of the genomic DNA of the target locus, sequencing of the mRNA transcript from the target locus, sequencing of the protein encoded at the target locus, or any of the known methods for identifying allelic variants at a given locus, such as those set forth in U.S. Patent Nos. 5,952,174, 5,846,710, 5,710,028 and 5,679,524, and those reviewed in Kwok, "High-throughput genotyping assay approaches," *Pharmacogenomics* 1(1):95-100 (2000), the disclosures of which are incorporated herein by reference. In addition, apparatus is now available commercially that permits the ready identification of allelic variants at a chosen target locus, such as the SniPer™ High Throughput SNP Scoring System (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and the SNPstream™ (Orchid Biosciences, Princeton, NJ, USA).

The cell for which the genotype is to be used as query can be a cultured cell or, alternatively, can be a noncultured cell derived directly from a eukaryotic organism. In the latter case, the genotype can be obtained, for example, from cells, such as circulating blood cells, that are replenishable *in vivo*. The cell for which the genotype is determined can be normally present in the eukaryotic organism or can be aberrant or otherwise diseased.

Usefully, the target locus genotype can be obtained from cells of a human being.

The query itself can include the entirety of the nucleic acid or protein sequence of the target locus, a portion of the nucleic acid or protein sequence of the target locus, even a single nucleotide or protein identifier and base or residue number that can serve as a unique identifier of the target locus genotype. Methods are well known in the bioinformatic arts for querying databases having sequence-related information.

The dataset to be queried includes correlations derived from at least five cells that are coisogenic at the target locus. Typically, the coisogenic cells will have been a cell collection according to the present invention.

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Where the cellular genotype used as query is derived from a human being, the above-described methods provide a streamlined approach to pharmacogenomic analysis.

An antecedent to traditional pharmacogenomic studies is the identification  
5 of a large number of naturally-occurring allelic variants, and correlation of the naturally-occurring alleles with naturally-occurring clinical phenotypes. Only then can a patient's genotype be used to predict the patient's probably clinical phenotype.

In contrast, the coisogenic collections of eukaryotic cells of the present invention allow all possible alleles readily to be constructed, and the resulting cellular  
10 phenotypes to be correlated with target locus genotype. Where the cellular phenotype can be correlated with the phenotype of the entire organism, as can readily be done with loci that affect responsiveness to xenobiotics, the dataset of correlated phenotypes can provide reliable phenotypic predictions, even for alleles that had not previously been identified within the natural population.

Thus, in certain particularly useful embodiments, the query genotype is  
15 from a human cell, and the target locus is selected from the group consisting of CYP1A2, CYP2C17, CYP2D6, CYP2E, CYP3A4, CYP4A11, CYP1B1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP11A, CYP2C19, CYP2F1, CYP2J2, CYP3A5, CYP3A7, CYP4B1, CYP4F2, CYP4F3, CYP6D1, CYP6F1, CYP7A1, CYP8, CYP11A, CYP11B1,  
20 CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, CYP51, ABCB1, ABCB4, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, MRP7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, EPHX1, EPHX2, LTA4H, TRAG3, GUSB, TMPT, BCRP, HERG, hKCNE2, UDP glucuronosyl transferase (UGT), sulfotransferase, sulfatase, glutathione S-transferase (GST) -alpha, glutathione S-transferase -mu, glutathione S-transferase -pi,  
25 ACE, and KCHN2, and the cellular phenotypic characteristic can usefully be cellular responsiveness to a xenobiotic; in such case, the prediction can be a prediction of an individual's potential responsiveness to that xenobiotic agent.

30 The following examples are offered for purpose of illustration and not by way of limitation.

EXAMPLE 1Coisogenic Eukaryotic Cell Collections  
Having Natural Allelic Variants of ABCB1 (MDR1)

Targeting oligos are used to create a cell collection coisogenic at the  
 5 human ABCB1 (MDR1) locus. The targeting oligonucleotides include terminal  
 modifications as set forth above, including at least one phosphorothiate linkage, and are  
 introduced in parallel into separate aliquots of HBL100 cells using standard techniques.  
 Potential cellular transformants are propagated *in vitro*, cloned, and clonal cell lines having  
 the desired targeted change identified by sequencing DNA amplified from the ABCB1 locus.

10 The targeting oligos have sequences (presented in Table 35, below)  
 designed to create natural allelic variants of the ABCB1 gene, creating a legacy-free,  
 perfectly coisogenic cell collection in which the naturally occurring alleles of ABCB1 are  
 presented on the identical genetic background of a human breast epithelial cell line.

The left-most column of the table identifies the alteration that converts the  
 15 wild type to the variant allele, at both the amino acid and the nucleotide level. At the amino  
 acid level, mutations are presented according to the following standard nomenclature. The  
 centered number identifies the position of the mutated codon in the protein sequence; to the  
 left of the number is the wild type residue and to the right of the number is the mutant  
 codon. At the nucleic acid level, the entire triplet of the wild type and mutated codons is  
 20 shown.

The middle column presents, for each alteration (mutation), four  
 oligonucleotides capable of changing the wild type sequence site-specifically to the  
 identified allelic variant.

All oligonucleotides are presented, per convention, in the 5' to 3'  
 25 orientation. The nucleotide that effects the change in the genome is underlined and  
 presented in bold.

The first of the four oligonucleotides for each mutation is a 121 nt  
 oligonucleotide centered about the altering ("repair") nucleotide. The second  
 oligonucleotide, its reverse complement, targets the opposite strand of the DNA duplex for  
 30 change ("repair"). The third oligonucleotide is the minimal 17 nt domain of the first

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oligonucleotide, also centered about the repair nucleotide. The fourth oligonucleotide is the reverse complement of the third, and thus represents the minimal 17 nt domain of the second.

The third column of the table presents the SEQ ID NO: of the respective  
5 targeting oligonucleotide.

Table 35 ABCB1 (MDR1) Targeting Oligos to Create Natural Alleles		
Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
10 Asn21Asp AAT-GAT	ATGGATCTTGAAGGGGA CCGCAATGGAGGAGCAA AGAAGAAGAACTTTTTTA AACTGAACGATAAAAGG TAACTAGCTTGTTTCATT TTCATAGTTTACATAGTT GCGAGATTTGAGTAAT	1
	ATTACTCAAATCTCGCAA CTATGTAACTATGAAAA TGAAACAAGCTAGTTACC TTTTATCGTTCAGTTTAA AAAAGTTCTTCTTCTTG CTCCTCCATTGCGGTCC CCTTCAAGATCCAT	2
	AACTGAACGATAAAAGG	3
	CCTTTTATCGTTCAGTT	4
Phe103Ser TTC-TCC	AAGAGACATAAATGGTAT GTTTGTGTTTGTGGTGGTC TAGGTGATATCAATGATA CAGGGTCTTCATGAAT CTGGAGGAAGACATGAC CAGGTAATTAGACATTCT CCTTACTATTGTAA	5

<b>Table 35</b> <b>ABCB1 (MDR1) Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	TTAACAATAGTAAGGAGA ATGTCTAATTACCTGGTC ATGTCTTCCTCCAGATTG ATGAAGGACCCTGTATC ATTGATATCACCTAGACC ACCACAAAACAAACATAC CATTTATGTCTCTT	6
	TACAGGGTCTTCATGA	7
	TCATGAAGGACCCTGTA	8
Phe103Leu TTC-CTC	AAAGAGACATAAATGGTA TGTTTGTTTTGTGGTGGT CTAGGTGATATCAATGAT ACAGGGCTCTTCATGAA TCTGGAGGAAGACATGA CCAGGTAATTAGACATTC TCCTTACTATTGTTA	9
	TAACAATAGTAAGGAGAA TGTCTAATTACCTGGTCA TGTCTTCCTCCAGATTCA TGAAGGCCCTGTATCA TTGATATCACCTAGACCA CCACAAAACAAACATACC ATTTATGTCTCTTT	10
	ATACAGGGCTCTTCATG	11
	CATGAAGAGCCCTGTAT	12
Gly185Val GGA-GTA	TTCTGACAATTATTCTA ACACTATCTGTTCTTTCA GTGATGTCTCCAAGATTA ATGAAGTAATTGGTGACA AAATTGGAATGTTCTTTC AGTCAATGGCAACATTTT TCACTGGGTTTAT	13

Table 35 ABCB1 (MDR1) Targeting Oligos to Create Natural Alleles		
Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
	ATAAACCCAGTGAAAAAT GTTGCCATTGACTGAAA GAACATTCCAATTTGTC ACCAATT <u>A</u> CTTCATTAAT CTTGGAGACATCACTGA AAGAACAGATAGTGTTA GAAATAATTGTCAGAA	14
	TAATGAAGT <u>A</u> ATTGGTG	15
	CACCAATT <u>A</u> CTTCATTA	16
Ser400Asn  AGT-AAT	AGAGTGGGCACAAACCA GATAATATTAAGGGAAAT TTGGAATTCAGAAATGTT CACTTCA <u>A</u> TTACCCATCT CGAAAAGAAGTTAAGGT ACAGTGATAAATGATTAA TCAACAATTAATCTA	17
	TAGATTAATTGTTGATTA ATCATTTATCACTGTACC TTAACTTCTTTTCGAGAT GGGTAAT <u>T</u> GAGTGAAC ATTTCTGAATTCCAAATT TCCCTTAATATTATCTGG TTTGTGCCCACTCT	18
	TCACTTCA <u>A</u> TTACCCAT	19
	ATGGGTAAT <u>T</u> GAGTGA	20
Val801Met  GTG-ATG	GGAGCTGAGAGTCTCAT AAACAGCTTTAAGGTAAT AAAATCATTTTCTGTGCC ACAGGAT <u>A</u> TGAGTTGGT TTGATGACCCTAAAACA CCACTGGAGCATTGACT ACCAGGCTCGCCAATG	21

**Table 35**  
**ABCB1 (MDR1) Targeting Oligos to Create Natural Alleles**

Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
	CATTGGCGAGCCTGGTA GTCAATGCTCCAGTGGT GTTTTAGGGTCATCAAA CCA <u>ACTCAT</u> ATCCTGTG GCACAGAAAATGATTTTA TTACCTTAAAGCTGTTTA TGAGACTCTCAGCTCC	22
	CACAGGAT <u>AT</u> AGATTGG	23
	CCA <u>ACTCAT</u> ATCCTGTG	24
Ile829Val ATA-GTA	AGCATGAGTTGTGAAGA TAATATTTTTAAAATTTCT CTAATTTGTTTTGTTTTG CAGGCT <u>G</u> TAGGTTCCAG GCTTGCTGTAATTACCCA GAATATAGCAAATCTTGG GACAGGAATAATTA	25
	TAATTATTCCTGTCCCAA GATTGCTATATTCTGGG TAATTACAGCAAGCCTG GAACCTA <u>C</u> AGCCTGCAA AACAAAACAAATTAGAGA AATTTTAAAAATATTATCT TCACAACTCATGCT	26
	TGCAGGCT <u>G</u> TAGGTTCC	27
	GGAACCTA <u>C</u> AGCCTGCA	28
Ser893Ala TCT-GCT	GTTGTTGAAATGAAAATG TTGTCTGGACAAGCACT GAAAGATAAGAAAGAAC TAGAAGGT <u>G</u> CTGGGAAG GTGAGTCAAATAAATAT GATTGATTAATTAAGTAG AGTAAAGTATTCTAAT	29



<b>Table 35</b> <b>ABCB1 (MDR1) Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	ATTAGAATACTTTACTCT ACTTAATTAATCAATCAT ATTTAGTTTGACTCACCT TCCCAGCACCTTCTAGTT CTTTCTTATCTTTCAGTG CTTGTCAGACAACATTT TCATTTCAACAAC	30
	TAGAAGGTGCTGGGAAG	31
	CTTCCCAGCACCTTCTA	32
Ser893Thr  TCT-ACT	GTTGTTGAAATGAAAATG TTGTCTGGACAAGCACT GAAAGATAAGAAAGAAC TAGAAGGTACTGGGAAG GTGAGTCAAATAAATAT GATTGATTAATTAAGTAG AGTAAAGTATTCTAAT	33
	ATTAGAATACTTTACTCT ACTTAATTAATCAATCAT ATTTAGTTTGACTCACCT TCCCAGTACCTTCTAGTT CTTTCTTATCTTTCAGTG CTTGTCAGACAACATTT TCATTTCAACAAC	34
	TAGAAGGTACTGGGAAG	35
	CTTCCCAGTACCTTCTA	36
Ala999Thr  GCC-ACC	TCAGCTGTTGTCTTTGGT GCCATGGCCGTGGGGC AAGTCAGTTCATTTGCTC CTGACTATACCAAAGCC AAAATATCAGCAGCCCA CATCATCATGATCATTGA AAAAACCCCTTTGATTG	37

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<b>Table 35</b> <b>ABCB1 (MDR1) Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	CAATCAAAGGGGTTTTTT CAATGATCATGATGATGT GGGCTGCTGATATTTTG GCTTTGGTATAGTCAGG AGCAAATGAACTGACTT GCCCCACGGCCATGGCA CCAAAGACAACAGCTGA	38
	CTGACTATACCAAAGCC	39
	GGCTTTGGTATAGTCAG	40
Gln1107Pro CAG-CCG	GATCTGTGAACCTTTGTT TTCAGCTGCTTGATGGC AAAGAAATAAAGCGACT GAATGTTCCGTGGCTCC GAGCACACCTGGGCATC GTGTCCCAGGAGCCCAT CCTGTTTGACTGCAGCA T	41
	ATGCTGCAGTCAAACAG GATGGGCTCCTGGGACA CGATGCCCAGGTGTGCT CGGAGCCACGGAACATT CAGTCGCTTTATTTCTTT GCCATCAAGCAGCTGAA ACAAGAGTTCACAGAT C	42
	GAATGTTCCGTGGCTCC	43
	GGAGCCACGGAACATTC	44

Aliquots of the coisogenic cell collection are thereafter separately contacted with a variety of chemotherapeutic agents presently used for, or contemplated for use in, treatment of breast adenocarcinoma, and alleles that increase or decrease sensitivity to the cytotoxic effects of the agents are identified.

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EXAMPLE 2Coisogenic Eukaryotic Cell Collections  
Having Natural Allelic Variants of CYP2D6

Targeting oligos are used to create a cell collection coisogenic at the  
5 human CYP2D6 locus.

The targeting oligonucleotides include terminal modifications as set forth  
above, including at least one phosphorothiate linkage, and are introduced in parallel into  
separate aliquots of HBL100 cells using standard techniques. Potential cellular  
transformants are propagated *in vitro*, cloned, and clonal cell lines having the desired  
10 targeted change identified by sequencing DNA amplified from the CYP2D6 locus.

The targeting oligos have sequences (presented in Table 36, below)  
designed to create natural allelic variants of the CYP2D6 gene, creating a legacy-free,  
perfectly coisogenic cell collection in which the naturally occurring alleles of CYP2D6 are  
presented on the identical genetic background of a human breast epithelial cell line.

15 The left-most column of the table identifies the alteration that converts the  
wild type to the variant allele, at both the amino acid and the nucleotide level. At the amino  
acid level, mutations are presented according to the following standard nomenclature. The  
centered number identifies the position of the mutated codon in the protein sequence; to the  
left of the number is the wild type residue and to the right of the number is the mutant  
20 codon. At the nucleic acid level, the entire triplet of the wild type and mutated codons is  
shown.

The middle column presents, for each alteration (mutation), four  
oligonucleotides capable of changing the wild type sequence site-specifically to the  
identified allelic variant.

25 All oligonucleotides are presented, per convention, in the 5' to 3'  
orientation. The nucleotide that effects the change in the genome is underlined and  
presented in bold.

The first of the four oligonucleotides for each mutation is a 121 nt  
oligonucleotide centered about the altering ("repair") nucleotide. The second  
30 oligonucleotide, its reverse complement, targets the opposite strand of the DNA duplex for  
change ("repair"). The third oligonucleotide is the minimal 17 nt domain of the first

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oligonucleotide, also centered about the repair nucleotide. The fourth oligonucleotide is the reverse complement of the third, and thus represents the minimal 17 nt domain of the second.

The third column of the table presents the SEQ ID NO: of the respective  
5 targeting oligonucleotide.

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
10 Val7Met GTG-ATG	GCCAGGTGTGTCCAGAGGAGCCCATTTGGTAGT GAGGCAGGTATGGGGCTAGAAGCACTGATGCCC CTGGCCGTGATAGTGGCCATCTCCTGCTCCTGG TGGACCTGATGCACCGGCGCC	45
	GGCGCCGGTGCATCAGGTCCACCAGGAGCAGGA AGATGGCCACTATCACGGCCAGGGGCATCAGTG CTTCTAGCCCCATACCTGCCTCACTACCAAATGG GCTCCTCTGGACACACCTGGC	46
	AAGCACTGATGCCCCCTG	47
	CAGGGGCATCAGTGCTT	48
Val11Met GTG-ATG	CAGAGGAGCCCATTTGGTAGTGAGGCAGGTATG GGGCTAGAAGCACTGGTGGCCCTGGCCATGATA GTGGCCATCTCCTGCTCCTGGTGGACCTGATGC ACCGGCGCCAACGCTGGGCTG	49
	CAGCCCAGCGTTGGCGCCGGTGCATCAGGTCCA CCAGGAGCAGGAAGATGGCCACTATCATGGCCA GGGGCACCAGTGCTTCTAGCCCCATACCTGCCTC ACTACCAAATGGGCTCCTCTG	50
	CCCTGGCCATGATAGTG	51
	CACTATCATGGCCAGGG	52
15 Arg26His CGC-CAC	TGGTGCCCTGGCCGTGATAGTGGCCATCTCCT GCTCCTGGTGGACCTGATGCACCGGCACCAACG CTGGGCTGCACGCTACCCACCAGGCCCTGCC ACTGCCCGGGCTGGGCAACCT	53

**Table 36**  
**CYP2D6 Targeting Oligos to Create Natural Alleles**

Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
	AGGTTGCCAGCCCGGGCAGTGGCAGGGGGCC TGGTGGGTAGCGTGCAGCCAGCGTTGGTGGCCG GTGCATCAGGTCCACCAGGAGCAGGAAGATGGC CACTATCACGGCCAGGGGCACCA	54
	GCACCGGCACCAACGCT	55
	AGCGTTGGTGGCGGTGC	56
Arg28Cys CGC-TGC	CCCCTGGCCGTGATAGTGGCCATCTTCCTGCTCC TGGTGGACCTGATGCACCGGCGCCAATGCTGGG CTGCACGCTACCCACCAGGCCCCCTGCCACTGC CCGGGCTGGGCAACCTGCTGC	57
	GCAGCAGGTTGCCAGCCCGGGCAGTGGCAGG GGGCCTGGTGGGTAGCGTGCAGCCAGCATTGG CGCCGGTGCATCAGGTCCACCAGGAGCAGGAAG ATGGCCACTATCACGGCCAGGGG	58
	GGCGCCAATGCTGGGCT	59
	AGCCCAGCATTGGCGCC	60
Pro34Ser CCA-TCA	GCCATCTTCCTGCTCCTGGTGGACCTGATGCACC GGCGCCAACGCTGGGCTGCACGCTACTCACCAG GCCCCCTGCCACTGCCCGGGCTGGGCAACCTGC TGCATGTGGACTTCCAGAACA	61
	TGTTCTGGAAGTCCACATGCAGCAGGTTGCCAG CCCGGGCAGTGGCAGGGGGCCTGGTGAGTAGC GTGCAGCCAGCGTTGGCGCCGGTGCATCAGGT CCACCAGGAGCAGGAAGATGGC	62
	CACGCTACTCACCAGGC	63
	GCCTGGTGAGTAGCGTG	64
Gly42Arg GGG-AGG	CTGATGCACCGGCGCCAACGCTGGGCTGCACGC TACCCACCAGGCCCCCTGCCACTGCCAGGCTG GGCAACCTGCTGCATGTGGACTTCCAGAACACAC CATACTGCTTCGACCAGGTGA	65

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	TCACCTGGTCTGAAGCAGTATGGTGTGTTCTGGAA GTCCACATGCAGCAGGTTGCCAGCCIGGGCAG TGGCAGGGGGCCTGGTGGGTAGCGTGCAGCCCA GCGTTGGCGCCGGTGCATCAG	66
	CACTGCCCAGGCTGGGC	67
	GCCCAGCCIGGGCAGTG	68
Ala85Val GCG-GTG	TCGGGGACGTGTTTCAGCCTGCAGCTGGCCTGGA CGCCGGTGGTCGTGCTCAATGGGCTGGTGGCCG TGCGCGAGGCGCTGGTGACCCACGGCGAGGACA CCGCCGACCGCCCGCCTGTGCC	69
	GGCACAGGCGGGCGGTGCGCGGTGTCCTCGCC GTGGGTCACCAGCGCCTCGCGCACGGCCACCAG CCCATTGAGCACGACCACGGCGTCCAGGCCAG CTGCAGGCTGAACACGTCCCCGA	70
	TGGGCTGGTGGCCGTGC	71
	GCACGGCCACCAGCCCA	72
Leu91Met CTG-ATG	CTGCAGCTGGCCTGGACGCCGGTGGTCGTGCTC AATGGGCTGGCGGCCGTGCGCGAGGCGATGGT GACCCACGGCGAGGACACCGCCGACCGCCCGC CTGTGCCCATCACCCAGATCCTGG	73
	CCAGGATCTGGGTGATGGGCACAGGCGGGCGGT CGGCGGTGTCCTCGCCGTGGGTCACCATCGCCT CGCGCACGGCCGCCAGCCATTGAGCACGACCA CCGGCGTCCAGGCCAGCTGCAG	74
	GCGAGGCGATGGTGACC	75
	GGTCACCATCGCCTCGC	76
5 His94Arg CAC-CGC	CCTGGACGCCGGTGGTCGTGCTCAATGGGCTGG CGGCCGTGCGCGAGGCGCTGGTGACCCGCGGC GAGGACACCGCCGACCGCCCGCCTGTGCCATC ACCCAGATCCTGGGTTTCGGGCC	77

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	GGCCCGAAACCCAGGATCTGGGTGATGGGCACA GGCGGGCGGTTCGGCGGTGTCTCGCCGCGGGT CACCAGCGCCTCGCGCACGGCCGCCAGCCCATT GAGCACGACCACCGGCGTCCAGG	78
	GGTGACCCGCGGCGAGG	79
	CCTCGCCGCGGGTCACC	80
Thr107Ile ACC-ATC	TGCGCGAGGCGCTGGTGACCCACGGCGAGGACA CCGCCGACCGCCCGCCTGTGCCCATCATCCAGA TCCTGGGTTTCGGGCGCGTTCCAAGGCAAGC AGCGGTGGGGACAGAGACAGAT	81
	ATCTGTCTCTGTCCCCACCGCTGCTTGCCTTGGG AACGCGGCCCCGAAACCCAGGATCTGGATGATGG GCACAGGCGGGCGGTTCGGCGGTGTCTCGCCG TGGGTCACCAGCGCCTCGCGCA	82
	GCCCATCATCCAGATCC	83
	GGATCTGGATGATGGGC	84
Val136Met GTG-ATG	CCCCAGGGGTGTTCTGGCGCGCTATGGGCCC GCGTGCGCGAGCAGAGGCGCTTCTCCATGTCC ACCTTGCGCAACTTGGGCCTGGGCAAGAAGTCG CTGGAGCAGTGGGTGACCGAGG	85
	CCTCGGTCACCACTGCTCCAGCGACTTCTTGCC CAGGCCCAAGTTGCGCAAGGTGGACATGGAGAA GCGCCTCTGCTCGCGCCACGCGGGCCCATAGCG CGCCAGGAACACCCCTGGGGG	86
	GCTTCTCCATGTCCACC	87
	GGTGGACATGGAGAAGC	88
5 Gln151Glu CAG-GAG	CAGAGGCGCTTCTCCGTGTCCACCTTGCGCAACT TGGGCCTGGGCAAGAAGTCGCTGGAGGAGTGGG TGACCGAGGAGGCCGCTGCCTTTGTCCGCCT TCGCCAACCACTCCGGTGGGT	89

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	ACCCACCGGAGTGGTTGGCGAAGGCGGCACAAA GGCAGGCGGCCTCCTCGGTACCCACT <u>C</u> CTCCA GCGACTTCTTGCCAGGCCCAAGTTGCGCAAGGT GGACACGGAGAAGCGCCTCTG	90
	CGCTGGAG <u>G</u> AGTGGGTG	91
	CACCCACT <u>C</u> CTCCAGCG	92
Asn166Asp AAC-GAC	AAGAAGTCGCTGGAGCAGTGGGTGACCGAGGAG GCCGCCTGCCTTTGTGCCGCCTTCGCC <u>G</u> ACCACT CCGGTGGGTGATGGGCAGAAGGGCACAAAGCGG GAACTGGGAAGGCGGGGGACG	93
	CGTCCCCCGCCTTCCCAGTTCCTCGCTTTGTGCCC TTCTGCCCATCACCCACCGGAGTGGT <u>C</u> GGCGAA GGCGGCACAAAGGCAGGCGGCCTCCTCGGTCAC CCTGCTCCAGCGACTTCTT	94
	CCTTCGCC <u>G</u> ACCACTCC	95
	GGAGTGGT <u>C</u> GGCGAAGG	96
Gly169Arg GGA-AGA	CTGGAGCAGTGGGTGACCGAGGAGGCCGCCTGC CTTTGTGCCGCCTTCGCCAACCACTCC <u>A</u> GTGGGT GATGGGCAGAAGGGCACAAAGCGGGAAGTGGGA AGGCGGGGACGGGGAAGGCG	97
	CGCCTTCCCCGTCCCCGCCTTCCCAGTTCCTCGC TTTGTGCCCTTCTGCCCATCACCCACT <u>I</u> GGAGTGG TTGGCGAAGGCGGCACAAAGGCAGGCGGCCTCC TCGGTCACCCACTGCTCCAG	98
	ACCACTCC <u>A</u> GTGGGTGA	99
	TCACCCACT <u>I</u> GGAGTGGT	100
5 Arg173Cys CGC-TGC	AGGCGGGGGACGGGGAAGGCGACCCCTTACCC GCATCTCCACCCCAAGGACGCCCCTTT <u>I</u> GCCCC AACGGTCTCTTGACAAAGCCGTGAGCAACGTGA TCGCCTCCCTCACCTGCGGGC	101



Table 36 CYP2D6 Targeting Oligos to Create Natural Alleles		
Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
	GCCCGCAGGTGAGGGAGGCGATCACGTTGCTCA CGGCTTTGTCCAAGAGACCGTTGGGGCAAAAGG GGCGTCCTGGGGGTGGGAGATGCGGGTAAGGG GTCGCCTTCCCCGTCCCCGCCT	102
	GCCCTTTTIGCCCCAAC	103
	GTTGGGGCAAAAGGGGC	104
Arg201His CGC-CAC	CCGTGAGCAACGTGATCGCCTCCCTCACCTGCG GGCGCCGCTTCGAGTACGACGACCCTCACTTCCT CAGGCTGCTGGACCTAGCTCAGGAGGGACTGAA GGAGGAGTCGGGCTTTCTGCG	105
	CGCAGAAAGCCCGACTCCTCCTCAGTCCCTCCT GAGCTAGGTCCAGCAGCCTGAGGAAGTGAGGGT CGTCGTA CTGAAGCGGCGCCCGCAGGTGAGGG AGGCGATCACGTTGCTCACGG	106
	CGACCCTCACTTCCTCA	107
	TGAGGAAGTGAGGGTCTG	108
Gly212Glu GGA-GAA	GGCGCCGCTTCGAGTACGACGACCCTCGCTTCC TCAGGCTGCTGGACCTAGCTCAGGAGGAACTGA AGGAGGAGTCGGGCTTTCTGCGCGAGGTGCGGA GCGAGAGACCGAGGAGTCTCTG	109
	CAGAGACTCCTCGGTCTCTCGCTCCGCACCTCGC GCAGAAAGCCCGACTCCTCCTCAGTTCCTCCTG AGCTAGGTCCAGCAGCCTGAGGAAGCGAGGGTC GTCGTA CTGAAGCGGCGCC	110
	TCAGGAGGAACTGAAGG	111
	CCTTCAGTTCCTCCTGA	112
5 Leu231Pro CTG-CCG	CAGGAGGGATTGAGACCCGTTCTGTCTGGTGTA GGTGCTGAATGCTGTCCCCGTCTCCCGCATATC CCAGCGCTGGCTGGCAAGGTCTACGCTTCCAAA AGGCTTTCCTGACCCAGCT	113

**Table 36**  
**CYP2D6 Targeting Oligos to Create Natural Alleles**

Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
	AGCTGGGTCAGGAAAGCCTTTTGAAGCGTAGG ACCTTGCCAGCCAGCGCTGGGATATGC <u>GGG</u> AGG ACGGGGACAGCATTTCAGCACCTACACCAGACAGA ACGGGGTCTCAATCCCTCCTG	114
	CGTCCTCC <u>C</u> GCATATCC	115
	GGATATGC <u>G</u> GGAGGACG	116
Ala237Ser GCT-TCT	CCGTTCTGTCTGGTGTAGGTGCTGAATGCTGTCC CCGTCCTCCTGCATATCCAGCGCTGTCTGGCAA GGTCCTACGCTTCCAAAAGGCTTTCTGACCCAG CTGGATGAGCTGCTAACTG	117
	CAGTTAGCAGCTCATCCAGCTGGGTCAGGAAAGC CTTTTGAAGCGTAGGACCTTGCCAG <u>A</u> CAGCGCT GGGATATGCAGGAGGACGGGGACAGCATTTCAGC ACCTACACCAGACAGAACGG	118
	CAGCGCTGTCTGGCAAG	119
	CTTGCCAG <u>A</u> CAGCGCTG	120
Arg296Cys CGC-TGC	GCTCTCGGCCCTGCTCAGGCCAAGGGGAACCTT GAGAGCAGCTTCAATGATGAGAACCTGTGCATAG TGGTGGCTGACCTGTTCTCTGCGGGGATGGTGA CCACCTCGACCACGCTGGCCT	121
	AGGCCAGCGTGGTCGAGGTGGTCACCATCCCGG CAGAGAACAGGTCAGCCACCACTATGC <u>A</u> CAGGTT CTCATCATTGAAGCTGCTCTCAGGGTTCCCCTTG GCCTGAGCAGGGCCGAGAGC	122
	AGAACCTGTGCATAGTG	123
	CACTATGC <u>A</u> CAGGTTCT	124
5 Ile297Leu ATA-CTA	CTCGGCCCTGCTCAGGCCAAGGGGAACCTGAG AGCAGCTTCAATGATGAGAACCTGCGC <u>C</u> TAGTGG TGGCTGACCTGTTCTCTGCCGGGATGGTGACCAC CTCGACCACGCTGGCCTGGG	125

Table 36 CYP2D6 Targeting Oligos to Create Natural Alleles		
Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
	CCCAGGCCAGCGTGGTCGAGGTGGTCACCATCC CGGCAGAGAACAGGTCAGCCACCACTAGGCGCA GGTTCTCATCATTGAAGCTGCTCTCAGGGTTCCC CTTGGCCTGAGCAGGGCCGAG	126
	ACCTGCGCCTAGTGGTG	127
	CACCACTAGGCGCAGGT	128
Ala300Gly GCG-GGT	CTCAGGCCAAGGGGAACCCTGAGAGCAGCTTCA ATGATGAGAACCTGCGCATAGTGGTGGGTGACCT GTTCTCTGCCGGGATGGTGACCACCTCGACCAC GCTGGCCTGGGGCCTCCTGCT	129
	AGCAGGAGGCCCCAGGCCAGCGTGGTCGAGGT GGTCACCATCCCGGCAGAGAACAGGTCACCCAC CACTATGCGCAGGTTCTCATCATTGAAGCTGCTC TCAGGGTTCCCCTTGGCCTGAG	130
	AGTGGTGGGTGACCTGT	131
	ACAGGTCA <del>C</del> CCACCACT	132
Asp301Asn GAC-AAC	CAGGCCAAGGGGAACCCTGAGAGCAGCTTCAAT GATGAGAACCTGCGCATAGTGGTGGCTAACCTGT TCTCTGCCGGGATGGTGACCACCTCGACCACGCT GGCCTGGGGCCTCCTGCTCA	133
	TGAGCAGGAGGCCCCAGGCCAGCGTGGTCGAG GTGGTCACCATCCCGGCAGAGAACAGGTTAGCC ACCACTATGCGCAGGTTCTCATCATTGAAGCTGC TCTCAGGGTTCCCCTTGGCCTG	134
	TGGTGGCTAACCTGTTC	135
	GAACAGGTTAGCCACCA	136
5 Ser311Leu TCG-TTG	ATGATGAGAACCTGCGCATAGTGGTGGCTGACCT GTTCTCTGCCGGGATGGTGACCACCTIGACCACG CTGGCCTGGGGCCTCCTGCTCATGATCCTACATC CGGATGTGCAGCGTGAGCC	137

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	GGCTCACGCTGCACATCCGGATGTAGGATCATGA GCAGGAGGCCCCAGGCCAGCGTGGTCAAGGTG GTCACCATCCCGGCAGAGAACAGGTCAGCCACC ACTATGCGCAGGTTCTCATCAT	138
	GACCACCTIGACCACGC	139
	GCGTGGTCAAGGTGGTC	140
His324Pro CAT-CCT	CTGCCGGGATGGTGACCACCTCGACCACGCTGG CCTGGGGCCTCCTGCTCATGATCCTACCTCCGGA TGTGCAGCGTGAGCCCATCTGGGAAACAGTGCA GGGGCCGAGGGAGGAAGGGTA	141
	TACCCCTTCCTCCCTCGGCCCTGCACTGTTTCCC AGATGGGCTCACGCTGCACATCCGGAGGTAGGA TCATGAGCAGGAGGCCCCAGGCCAGCGTGGTCG AGGTGGTCACCATCCCGGCAG	142
	GATCCTACCTCCGGATG	143
	CATCCGGAGGTAGGATC	144
Pro325Leu CCG-CTG	CCGGGATGGTGACCACCTCGACCACGCTGGCCT GGGGCCTCCTGCTCATGATCCTACATCTGGATGT GCAGCGTGAGCCCATCTGGGAAACAGTGCAAGG GCCGAGGGAGGAAGGGTACAG	145
	CTGTACCCCTTCCTCCCTCGGCCCTGCACTGTTT CCCAGATGGGCTCACGCTGCACATCCAGATGTAG GATCATGAGCAGGAGGCCCCAGGCCAGCGTGGT CGAGGTGGTCACCATCCCGG	146
	CCTACATCTGGATGTGC	147
	GCACATCCAGATGTAGG	148
5 Val338Met GTG-ATG	TGCTGACCCATTGTGGGGACGCATGTCTGTCCAG GCCGTGTCCAACAGGAGATCGACGACATGATAG GGCAGGTGCGGCGACCAGAGATGGGTGACCAG GCTCACATGCCCTACACCACTG	149

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Table 36 CYP2D6 Targeting Oligos to Create Natural Alleles		
Allelic Variation	Sequence of Targeting Oligos	SEQ ID NO:
	CAGTGGTGTAGGGCATGTGAGCCTGGTCACCCAT CTCTGGTCGCCGCACCTGCCCTATCA <u>T</u> GTCGTCTG ATCTCCTGTTGGACACGGCCTGGACAGACATGCG TCCCCACAATGGGTCAGCA	150
	TCGACGAC <u>A</u> TGATAGGG	151
	CCCTATCA <u>T</u> GTCGTCTGA	152
Arg343Gly CGG-GGG	GGGACGCATGTCTGTCCAGGCCGTGTCCAACAG GAGATCGACGACGTGATAGGGCAGGTG <u>G</u> GGCGA CCAGAGATGGGTGACCAGGCTCACATGCCCTACA CCTGCGCGTGATTCATGAGG	153
	CCTCATGAATCACGGCAGTGGTGTAGGGCATGTG AGCCTGGTCACCCATCTCTGGTCGCC <u>C</u> CACCTGC CCTATCACGTCGTCTGATCTCCTGTTGGACACGGC CTGGACAGACATGCGTCCC	154
	GGCAGGTG <u>G</u> GGCGACCA	155
	TGGTCGCC <u>C</u> CACCTGCC	156
Arg365His CGC-CAC	CAGAGATGGGTGACCAGGCTCACATGCCCTACAC CACTGCCGTGATTCATGAGGTGCAGC <u>A</u> CTTTGGG GACATCGTCCCCCTGGGTGTGACCCATATGACAT CCCGTGACATCGAAGTACA	157
	TGTA <u>C</u> TTGATGTCACGGGATGTCATATGGGTCA CAGGAGGGGACGATGTCCCAAAG <u>T</u> GCTGCA CCTCATGAATCACGGCAGTGGTGTAGGGCATGTG AGCCTGGTCACCCATCTCTG	158
	GGTGCAGC <u>A</u> CTTTGGGG	159
	CCCCAAAG <u>T</u> GCTGCACC	160
5 Ile369Thr ATC-ACC	ACCAGGCTCACATGCCCTACACCACTGCCGTGAT TCATGAGGTGCAGCGCTTTGGGGACA <u>C</u> CGTCCC CCTGGGTGTGACCCATATGACATCCCGTGACATC GAAGTACAGGGCTTCCGCAT	161

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	ATGCGGAAGCCCTGACTTCGATGTCACGGGATG TCATATGGGTACACCCAGGGGACGGTGTCCC CAAAGCGCTGCACCTCATGAATCACGGCAGTGGT GTAGGGCATGTGAGCCTGGT	162
	TGGGGACACCGTCCCCC	163
	GGGGGACGGTGTCCCCA	164
Gly373Ser GGT-AGT	ATGCCCTACACCACTGCCGTGATTCATGAGGTGC AGCGCTTTGGGGACATCGTCCCCCTGAGTGTGA CCCATATGACATCCCGTGACATCGAAGTACAGGG CTTCCGCATCCCTAAGGTAG	165
	CTACCTTAGGGATGCGGAAGCCCTGACTTCGAT GTCACGGGATGTCATATGGGTACACTCAGGGG GACGATGTCCCCAAAGCGCTGCACCTCATGAATC ACGGCAGTGGTGTAGGGCAT	166
	TCCCCCTGAGTGTGACC	167
	GGTCACACTCAGGGGGA	168
Val374Met GTG-ATG	CCCTACACCACTGCCGTGATTCATGAGGTGCAGC GCTTTGGGGACATCGTCCCCCTGGGTATGACCCA TATGACATCCCGTGACATCGAAGTACAGGGCTTC CGCATCCCTAAGGTAGGCC	169
	GGCCTACCTTAGGGATGCGGAAGCCCTGACTTC GATGTCACGGGATGTCATATGGGTCAIACCCAGG GGGACGATGTCCCCAAAGCGCTGCACCTCATGAA TCACGGCAGTGGTGTAGGG	170
	CCCTGGGTATGACCCAT	171
	ATGGGTCAIACCCAGGG	172
5 Glu410Lys GAG-AAG	GCCCAGGGAACGACACTCATCACCAACCTGTCAT CGGTGCTGAAGGATGAGGCCGTCTGGAAGAAGC CCTTCCGCTTCCACCCCGAACACTTCCTGGATGC CCAGGGCCACTTTGTGAAGC	173

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	GCTTCACAAAGTGGCCCTGGGCATCCAGGAAGT GTTCTGGGGTGGAAAGCGGAAGGGCTTCTTCCAGA CGGCCTCATCCTTCAGCACCGATGACAGGTTGGT GATGAGTGTCTGTTCCCTGGGC	174
	CCGTCTGGAAGAAGCCC	175
	GGGCTTCTTCCAGACGG	176
Glu418Gln GAA-CAA	AACCTGTCATCGGTGCTGAAGGATGAGGCCGTCT GGGAGAAGCCCTTCCGCTTCCACCCC <del>C</del> AACACTT CCTGGATGCCAGGGCCACTTTGTGAAGCCGGA GGCCTTCCTGCCTTTCTCAG	177
	CTGAGAAAGGCAGGAAGGCCTCCGGCTTCACAA AGTGGCCCTGGGCATCCAGGAAGTGTG <del>G</del> GGGGT GGAAGCGGAAGGGCTTCTCCAGACGGCCTCAT CCTTCAGCACCGATGACAGGTT	178
	TCCACCCC <del>C</del> AACACTTC	179
	GAAGTGTTG <del>G</del> GGGGTGGA	180
Leu421Pro CTG-CCG	CGGTGCTGAAGGATGAGGCCGTCTGGGAGAAGC CCTTCCGCTTCCACCCCGAACACTTCC <del>C</del> GGATGC CCAGGGCCACTTTGTGAAGCCGGAGGCCTTCCT GCCTTTCTCAGCAGGTGCCTG	181
	CAGGCACCTGCTGAGAAAGGCAGGAAGGCCTCC GGCTTCACAAAGTGGCCCTGGGCATCC <del>G</del> GGAAG TGTTCTGGGGTGGAAAGCGGAAGGGCTTCTCCAG ACGGCCTCATCCTTCAGCACCG	182
	ACACTTCC <del>C</del> GGATGCCC	183
	GGGCATCC <del>G</del> GGAAGTGT	184
5 Arg440His CGC-CAC	TCTTGCAGGGGTATCACCCAGGAGCCAGGCTCA CTGACGCCCTCCCTCCCCACAGGCC <del>A</del> CCGTG CATGCCTCGGGGAGCCCCTGGCCCGCATGGAGC TCTTCCTCTTCTCACCTCCCT	185

<b>Table 36</b> <b>CYP2D6 Targeting Oligos to Create Natural Alleles</b>		
<b>Allelic Variation</b>	<b>Sequence of Targeting Oligos</b>	<b>SEQ ID NO:</b>
	AGGGAGGTGAAGAAGAGGAAGAGCTCCATGCGG GCCAGGGGCTCCCCGAGGCATGCACGGTGGCCT GTGGGGAGGGGAGGGGCGTCAGTGAGCCTGGC TCCTGGGTGATACCCCTGCAAGA	186
	CACAGGCCACCGTG CAT	187
	ATGCACGGTGGCCTGTG	188
Met451Ile ATG-ATA	TGACGCCCCTCCCCTCCCCACAGGCCGCCGTGC ATGCCTCGGGGAGCCCCTGGCCCGCATAGAGCT CTTCCTCTTCTTACCTCCCTGCTGCAGCACTTCA GCTTCTCGGTGCCCACTGGA	189
	TCCAGTGGGCACCGAGAAGCTGAAGTGTGTCAG CAGGGAGGTGAAGAAGAGGAAGAGCTCTATGCG GGCCAGGGGCTCCCCGAGGCATGCACGGCGGC CTGTGGGGAGGGGAGGGGCGTCA	190
	GCCCGCATAGAGCTCTT	191
	AAGAGCTCTATGCGGGC	192
Ser486Thr AGC-ACC	TCTCGGTGCCCCACTGGACAGCCCCGGCCAGCC ACCATGGTGTCTTTGCTTTCCTGGTGACCCCATC CCCCTATGAGCTTTGTGCTGTGCCCCGCTAGAAT GGGTACCTAGTCCCCAGCC	193
	GGCTGGGGACTAGGTACCCATTCTAGCGGGGC ACAGCACAAAGCTCATAGGGGGATGGGGTCACC AGGAAAGCAAAGACACCATGGTGGCTGGGCCGG GGCTGTCCAGTGGGCACCGAGA	194
	CCTGGTGACCCCATCCC	195
	GGGATGGGGTCACCAGG	196

- 5 Aliquots of the coisogenic cell collection are thereafter separately contacted with a variety of chemotherapeutic agents presently used for, or contemplated for use in, treatment of breast adenocarcinoma, and alleles that increase or decrease sensitivity to the cytotoxic effects of the agents are identified.



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All patents, patent publications, and other published references mentioned herein are hereby incorporated by reference in their entireties as if each had been individually and specifically incorporated by reference herein. While preferred illustrative  
5 embodiments of the present invention are described, one skilled in the art will appreciate that the present invention can be practiced by other than the described embodiments, which are presented for purposes of illustration only and not by way of limitation. The present invention is limited only by the claims that follow.

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What is claimed is:

1. A collection of cultured cells, comprising:  
at least 5 genotypically distinct cells,  
wherein each of said at least 5 genotypically distinct cells is coisogenic with respect to the others of said at least 5 genotypically distinct cells at a target locus common thereamong, and  
wherein each of said at least 5 genotypically distinct cells can be separately assayed.
2. The cell collection of claim 1, comprising at least 10 genotypically distinct cells.
3. The cell collection of claim 2, comprising at least 25 genotypically distinct cells.
4. The cell population of any one of claims 1 - 3, wherein said cells are mammalian cells.
5. The cell population of claim 4, wherein said mammalian cells are human cells.
6. The cell population of claim 4, wherein said mammalian cells are rodent cells.
7. The cell population of claim 6, wherein said rodent cells are mouse cells.
8. The cell population of any one of claims 1 - 3, wherein said cells are yeast cells.

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9. The cell population of any one of claims 1 - 3, wherein said cells are plant cells.

10. The cell collection of any one of claims 1 - 9, wherein each of said genotypically distinct cells is disposed in fluid noncommunication with each of the other of said genotypically distinct cells.

11. The cell collection of claim 10, wherein each of said genotypically distinct cells is spatially addressable.

12. The cell collection of any one of claims 1 - 11, wherein said genotypically distinct cells collectively include each of the 20 natural amino acids at a single residue encoded at the target locus.

13. The cell collection of any one of claims 1 - 12, wherein said genotypically distinct cells collectively include a predetermined amino acid at each residue encoded after the initiator methionine at the target locus.

14. The cell collection of any one of claims 1 - 13, wherein said genotypically distinct cells collectively include at least one naturally occurring allele of the target locus.

15. The cell collection of claim 14, wherein said genotypically distinct cells collectively include a plurality of naturally occurring alleles of the target locus.

16. The cell collection of any one of claims 1 - 15, wherein said genotypically distinct cells further comprise a common selectable marker at a genomic locus different from said target locus.

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17. The cell collection of any one of claims 1 - 16, wherein said genotypically distinct cells each further comprises a marker unique to said genotypically distinct cell, said marker being at a locus different from said target locus.

18. The cell collection of any one of claims 1 - 17, wherein said target locus is selected from the group consisting of: CYP1A2, CYP2C17, CYP2D6, CYP2E, CYP3A4, CYP4A11, CYP1B1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP11A, CYP2C19, CYP2F1, CYP2J2, CYP3A5, CYP3A7, CYP4B1, CYP4F2, CYP4F3, CYP6D1, CYP6F1, CYP7A1, CYP8, CYP11A, CYP11B1, CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, CYP51, ABCB1, ABCB4, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, MRP7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, EPHX1, EPHX2, LTA4H, TRAG3, GUSB, TMPT, BCRP, HERG, hKCNE2, UDP glucuronosyl transferase (UGT), sulfotransferase, sulfatase, glutathione S-transferase (GST) -alpha, glutathione S-transferase -mu, glutathione S-transferase -pi, ACE, and KCHN2.

19. The cell collection of claim 18, wherein said target locus is ABCB1.

20. The cell collection of any one of claims 1 - 19, wherein said coisogenic cells are legacy-free.

21. The cell collection of any one of claims 1 - 20, wherein said coisogenic cells are exceptionally coisogenic.

22. The cell collection of any one of claims 1 - 21, wherein said coisogenic cells are perfectly coisogenic.

23. A kit, comprising:  
at least five genotypically distinct cells, said cells contained within separate, structurally discrete, fluidly noncommunicating containers, wherein each of said at least 5 genotypically distinct cells is coisogenic with respect the others of said at least 5 genotypically distinct cells at a target locus common thereamong;

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wherein said at least five structurally discrete containers are commonly packaged.

24. The kit of claim 23, wherein said at least five genotypically distinct, commonly packaged, cells constitute a coisogenic cell collection according to any one of claims 1 - 22.

25. The kit of claim 23 or claim 24, further comprising:  
a computer readable medium, said computer readable medium containing a dataset that describes the target locus genotype of each of said genotypically distinct cells.

26. A method of making a coisogenic cell collection, the method comprising:  
collecting at least 5 genotypically distinct cells, each of said genotypically distinct cells being coisogenic with respect to the others of said at least 5 genotypically distinct cells at a target locus common thereamong, into a collection in which each of said at least 5 genotypically distinct cells can be separately assayed.

27. The method of claim 26, further comprising the antecedent step of:  
engineering, into at least four of said at least five cultured cells, said cells having derived from a common eukaryotic ancestor cell, a genomic sequence alteration at a target locus common thereamong, said sequence alterations being sufficient to cause at least five distinct protein sequences collectively to be encoded by said cells at said target locus.

28. The method of claim 27, wherein said engineering is effected by introducing a targeting oligonucleotide into each of said at least four cultured cells.

29. The method of claim 27, wherein said engineering step is effected by introducing into each of said at least four cultured cells a recombination-competent

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substrate into which said genomic sequence alteration has previously been introduced using a targeting oligonucleotide.

30. A kit, comprising:  
at least four targeting oligonucleotides of distinct sequence; and  
a eukaryotic cell,  
wherein said oligonucleotides are sufficient for use in the method of claim 28 to create the cell collections of any of claims 1 - 22 from said eukaryotic cell.

31. A method of identifying genotypes of a target locus that alter a cellular phenotype, comprising:  
assaying each genotypically distinct cell of a coisogenic cell collection for a common phenotypic characteristic, wherein said genotypically distinct cells are coisogenic at said target locus, and wherein said collection is a coisogenic cell collection according to any one of claims 1 - 22;  
identifying from said assay results at least one cell having an altered phenotypic characteristic; and  
correlating, for at least said at least one cell with altered phenotypic characteristic, the results of said phenotypic assay with said cell's target locus genotype,  
the correlation of phenotypic assay results with target locus genotype  
identifying genotypes of said target locus that alter said cellular phenotype.

32. The method of claim 31, wherein said phenotypic characteristic is responsiveness of said cell to a xenobiotic.

33. The method of claim 31 or claim 32, further comprising the antecedent step of:  
contacting said coisogenic cell collection with a xenobiotic.

34. The method of any of claims 31 - 33, wherein said target locus is selected from the group consisting of: CYP1A2, CYP2C17, CYP2D6, CYP2E, CYP3A4,

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CYP4A11, CYP1B1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP11A, CYP2C19, CYP2F1, CYP2J2, CYP3A5, CYP3A7, CYP4B1, CYP4F2, CYP4F3, CYP6D1, CYP6F1, CYP7A1, CYP8, CYP11A, CYP11B1, CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, CYP51, ABCB1, ABCB4, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, MRP7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, EPHX1, EPHX2, LTA4H, TRAG3, GUSB, TMPT, BCRP, HERG, hKCNE2, UDP glucuronosyl transferase (UGT), sulfotransferase, sulfatase, glutathione S-transferase (GST) -alpha, glutathione S-transferase -mu, glutathione S-transferase -pi, ACE, and KCHN2.

35. The method of any one of claims 31 - 34, further comprising the step, after said correlating, of:

collecting said correlations into at least one dataset.

36. The method of claim 34, wherein said dataset is recorded on a computer-readable medium.

37. A method of predicting a phenotypic characteristic of a cell based upon its genotype at a target locus, comprising:

using said cell's genotype at said target locus, or a unique identifier thereof, as a query to retrieve from a dataset data that report a correlated phenotypic characteristic, wherein said dataset includes correlations of a phenotypic characteristic with target locus genotype for at least five cells that are coisogenic at said target locus, said retrieved phenotypic characteristic providing a prediction of said cell's phenotypic characteristic.

38. The method of claim 37, wherein said at least five cells that are coisogenic at said target locus genotype are a cell collection according to any one of claims 1 - 22.

39. The method of claim 37 or claim 38, wherein said target locus is selected from the group consisting of: CYP1A2, CYP2C17, CYP2D6, CYP2E, CYP3A4,

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CYP4A11, CYP1B1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP11A, CYP2C19, CYP2F1, CYP2J2, CYP3A5, CYP3A7, CYP4B1, CYP4F2, CYP4F3, CYP6D1, CYP6F1, CYP7A1, CYP8, CYP11A, CYP11B1, CYP11B2, CYP17, CYP19, CYP21A2, CYP24, CYP27A1, CYP51, ABCB1, ABCB4, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, MRP7, ABCC8, ABCC9, ABCC10, ABCC11, ABCC12, EPHX1, EPHX2, LTA4H, TRAG3, GUSB, TMPT, BCRP, HERG, hKCNE2, UDP glucuronosyl transferase (UGT), sulfotransferase, sulfatase, glutathione S-transferase (GST) -alpha, glutathione S-transferase -mu, glutathione S-transferase -pi, ACE, and KCHN2.